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Exogenous Corticosteroids and Dog Behaviour

L. Notari

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Exogenous Corticosteroids and Dog Behaviour

Author

Lorella Notari

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Supervisor
Professor Daniel Mills

Second Supervisor
Dr. Oliver Burman

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To my family, Giuseppe, Francesco and Margherita

Preface

Some of the work contained in this thesis has been published in two peer-reviewed journals (Appendix H), presented in two peer-reviewed International and European conferences as well as in one invited talk in UK and in two invited talks in Italy.

Journals

Notari L., Mills D. (2011). Possible behavioural effects of exogenous corticosteroids on dog behaviour: a preliminary investigation. *Journal of Veterinary Behaviour: Clinical Applications and Research*. Vol 6, Issue 6, Pages 321-327.

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Notari L. Glucocorticoidi esogeni e alterazioni comportamentali nel cane (Exogenous corticosteroids and behavioural changes in dogs). AIVPA National Veterinary Meeting - Problema dermatologico o comportamentale? - Varese 17 November 2012.

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Abstract

Arousal and distress are considered important factors when dogs show problematic behaviours and the crucial role of hormones and neurotransmitters involved in stress responses is widely recognized.

Corticosteroids are important players in stress responses and, along with other hormones and neurotransmitters, contribute to the onset of both physiological and behavioural changes that can be either normal and adaptive or excessive and maladaptive depending on several factors. A literature review revealed that exogenous corticosteroids have been reported to change behaviour in human beings and laboratory animals but no data were available as far as similar effects in dogs.

The aim of this research was to identify possible behavioural changes in dogs treated with corticosteroids. In the first study, the perception of behavioural changes in dogs during corticosteroid therapy was investigated through semi-structured open interviews of the owners of 31 dogs of different breeds, genders, and ages. All dogs had received corticosteroid therapies in the past six months. Owners were asked to describe their dog's behaviours both on and off corticosteroid therapy.

Eleven owners reported behavioural changes in their dogs; nine dogs were reported to show more than one behavioural change. Six dogs reportedly showed nervousness and/or restlessness, three showed an increase in startle responses, three showed food guarding, two showed a decrease in their activity level, three showed an increase in avoidance responses, four showed irritable aggression, and two dogs increased barking. Semi-structured interviews can be useful preliminary tools for the identification of areas of future investigation, and the outcomes of the interviews were then used to investigate more rigorously the possible relationship between these signs and corticosteroid use in dogs.

In the second study 99 dog owners were asked to complete a 12 item questionnaire, developed following the results of the previous survey. Owners were asked to eval-

uate their dogs' behaviour on and off therapy, using a seven point scale. A sample of owners whose dogs were receiving treatment for dermatological, orthopaedic or other conditions completed the survey. The survey was completed by 44 dog owners with animals receiving treatment with a range of corticosteroid preparations (mainly prednisolone and methylprednisolone) and 54 dog owners with dogs receiving treatment with other drugs, mainly antibiotics and non-steroidal anti-inflammatory drugs. Dogs under corticosteroid treatment were reported to be significantly less playful, more nervous/restless, more fearful/less confident, more aggressive in the presence of food, more prone to barking, more prone to startle, more prone to reacting aggressively when disturbed, and more prone to avoiding people or unusual situations. The last part of this study involved behavioural tests of dogs.

Eleven "treatment" dogs were then tested twice: before and during corticosteroid treatment with either methyl-prednisolone or prednisolone to assess their sensitivity to a potentially aversive stimulus. Eleven control dogs, not receiving corticosteroid therapy, were also tested at the same time intervals in the same environment. Dogs were exposed to a brief dog growl while they explored some bowls containing food and their behaviour was video recorded. Treatment dogs investigated the area for significantly less time and ate significantly less food in the second test trial when on corticosteroid compared to control dogs.

In final study, exploring relationships between corticosteroid therapy and dogs with behaviour problems, a review of the caseload of the author of 345 dogs reported for behaviour and management problems was analyzed. It was found that 16 % of them had a history of previous treatments with corticosteroids.

Previous treatment with corticosteroid was found to be significantly associated with negative affective states.

These results support earlier preliminary findings concerning possible adverse behavioural side effects following the use of corticosteroids in dogs, and the possible need for concomitant behavioural advice when these drugs are used in general veterinary practice.

Sommario

I livelli di eccitazione e stress sono considerati importanti fattori quando i cani mostrano comportamenti problematici e il ruolo cruciale di ormoni e neurotrasmettitori coinvolti nelle risposte allo stress è stato ampiamente riconosciuto. I corticosteroidi sono protagonisti importanti nelle risposte da stress e, insieme ad altri ormoni e neurotrasmettitori, contribuiscono all'instaurarsi di cambiamenti sia fisiologici sia comportamentali che possono essere normali e adattativi oppure eccessivi e non adattativi a seconda di diversi fattori. Una approfondita rassegna della letteratura scientifica ha mostrato che i corticosteroidi esogeni sono stati segnalati come possibili fattori che cambiano il comportamento negli esseri umani e negli animali da laboratorio ma non sono stati trovati dati disponibili per quanto riguarda simili effetti nei cani.

Lo scopo di questa ricerca era di identificare possibili cambiamenti comportamentali nei cani trattati con corticosteroidi usando diverse metodologie .

Nella prima fase di questa ricerca, le percezioni di cambiamenti comportamentali nei cani in corso di terapia con corticosteroidi è stata investigata attraverso interviste aperte semi-strutturate ai proprietari di 31 cani di diverse razze, sesso e età. Tutti i cani avevano assunto terapie corticosteroidiche negli ultimi sei mesi.

Ai proprietari era stato chiesto di descrivere il comportamento dei loro cani sia durante la terapia che senza terapia.

Nel complesso, 11 proprietari riferirono cambiamenti nel comportamento dei loro cani: nove proprietari riferirono che i loro cani avevano mostrato più di un cambiamento comportamentale. Sei cani avevano mostrato nervosismo o agitazione, 3 avevano mostrato un aumento nelle risposte di soprassalto, tre avevano mostrato la tendenza a difendere il cibo aggressivamente, due avevano mostrato una diminuzione nel livello di attività, tre avevano mostrato un aumento delle risposte di evitamento, quattro avevano mostrato aggressività per irritazione e due un aumento dell'abbaio.

Le interviste semi-strutturate possono essere utili come strumenti per identificare le

aree da investigare nel futuro e i risultati delle interviste riportati in questa prima fase della ricerca sono stati poi usati per investigare in maniera più rigorosa le possibili relazioni tra questi segni e l'uso di corticosteroidi nei cani.

Nel secondo stadio della ricerca 99 proprietari di cani hanno compilato un questionario con 12 domande che etè stato sviluppato a partire dei risultati dello studio precedente. Ai proprietari è stato chiesto di valutare il comportamento dei loro cani in terapia e senza terapia usando una scala di sette punti. Un campione di proprietari di cani che avevano ricevuto una terapia per problemi dermatologici, ortopedici e altri problemi hanno completato lo studio. Lo studio è stato completato da 44 proprietari di cani che avevano ricevuto terapie costituite da diversi tipi di corticosteroidi (soprattutto prednisolone e metilprednisolone) e 54 proprietari di cani che avevano ricevuto terapie costituite da altri farmaci, soprattutto antibiotici e antiinfiammatori non steroidei. Una analisi multivariata General Linear Model (GLM) e GLM con correzione post hoc per test multipli ha rivelato che i cani in terapia con corticosteroidi sono stati descritti dai proprietari come significativamente meno giocosi, più nervosi/agitati, più paurosi/meno sicuri di sé, più aggressive in presenza di cibo, più portati ad abbaiare, più portati a mostrare reazioni di soprassalto, più portati a reagire aggressivamente quando disturbati e più portati a evitare persone o situazioni inusuali.

Nell'ultima parte di questa ricerca sono stati svolti test comportamentali su alcuni cani.

Undici cani “in trattamento” sono stati testati due volte: prima e durante la terapia con corticosteroidi attuata con metilprednisolone o prednisolone per valutarne la sensibilità a uno stimolo potenzialmente avversativo. Undici cani di controllo sono stati testati allo stesso intervallo di tempo e nello stesso contesto . I cani sono stati esposti a un breve ringhio mentre esploravano alcune ciotole contenenti cibo e il loro comportamento è stato videoregistrato. I cani del gruppo “trattamento” hanno investigato l'area del test per un tempo significativamente minore e hanno mangiato un numero significativamente minore di bocconi di cibo nel secondo test, quando erano in terapia, diversamente dai cani di controllo.

Per dare una dimensione dell'importanza di questi risultati, una casistica di 345 cani portati alla visita per problemi di comportamento e di gestione è stata analizzata ed è stato trovato che il 16% di questi cani aveva avuto una storia di precedenti terapie con corticosteroidi. Una significativa correlazione è stata trovata tra precedenti terapie con corticosteroidi e stati affettivi negativi.

Questi dati supportano i dati preliminari riguardo a possibili effetti collaterali comportamentali a seguito di impiego di corticosteroidi nei cani e la necessità di fornire indicazione comportamentali quando questi farmaci vengono impiegati nella pratica clinica veterinaria.

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List of Abbreviations

ACTH	AdrenoCorticoTrophic Hormone
AVP	Arginine VasoPressine
CNS	Central Nervous System
CRF	Corticosteroid Releasing Factor
CRH	Corticosteroid Releasing Hormone
CT	Computerized Tomography
GABA	Gamma Amino Butyrric Acid
GCs	GlucoCorticoids
GLM	General Linear Model
GRs	Glucocorticoid Receptors
GZLM	GeneraliZed Linear Model
HPA	Hyipothalamic Adrenal Axis
LC	Locus Coeruleus
LTP	Long Term Potentiation
MRI	Magnetic Resonance Imaging
MRs	Mineralocorticoid Receptor
NE	NorEpinephrine (Noradrenaline)

LIST OF TABLES

NSAIDs	Non Steroid Anti-Inflammatory Drugs
PFC	PreFrontal Cortex
PVN	ParaVentricular Nucleus
THIN	The Health Improvement Network

Chapter 1

General Introduction

1.1 Introduction

Increasing attention is being given to the prevention and treatment of dog behaviour problems (Adams and Clark, 1989; Herron *et al.*, 2008; Ibáñez and Anzola, 2009; Seksel and Lindeman, 2001; Sherman and Mills, 2008; Takeuchi *et al.*, 2001; Wright and Nesselrote, 1987).

The influence of environmental factors and health problems on the onset of dog behaviour problems has been dealt with by many authors and the roles of stress and anxiety, concepts that are often used as if they are interchangeable (although anxiety is only a form of stressor that a dog may be subjected to), have been recognized in both the onset and continuation of problematic behaviours in companion animals (Aronson and Dodds, 2005; Casey *et al.*, 2013; Fatjó *et al.*, 2002; Galac and Knol, 1997; Kobelt *et al.*, 2007; O’Farrell, 1997; Westgarth *et al.*, 2010). It has been stated that most dog behaviour problems are “stress related” and cortisol level is often used as a behavioural indicator of stress conditions in dogs (Beerda *et al.*, 2000; Ottenheimer Carrier *et al.*, 2013). The term “stress” is certainly overused and often poorly defined or oversimplified. Therefore the main aim of the following introductory part of this chapter is to show how such a common word might refer to very complex mechanisms in the brain, often mediated through a cortisol response. The experimental research concerns the effects of corticosteroid drugs on dog behaviour and so it is important to put the function of this chemical in both a biological and historical context, as ideas have changed since its discovery in the last century.

1.2 Stress And The Hypothalamic-Pituitary-Adrenal Axis (HPA)

The words “stress” and “distress” are used to describe emotionally and physiologically challenging experiences that result in change in both behavioural and physical signs with the brain being the target and the master of stress responses. Positive adaptation to challenges was called “eustress” and the negative adaptation to challenges was called “distress”. Positive stress (eustress) is generally short term, perceived as within the coping ability of an individual and it improves adaptation (Lazarus, 1993). Distress can be a short or long term challenging experience that has negative implications for the individual’s adaptation and welfare (Selye, 1974). Different

stress responses are also performed as a consequence of challenges of different intensity, duration and frequency of exposure. A single exposure of a single intense challenge elicits what is called an acute stress response. The exposure to challenges repeated over time or long term exposure to stressful events can lead to chronic stress (McEwen, 2004). Hans Selye is widely recognized as the pioneer investigator into the non-specific physiological response of the body to any demand for change. In 1936 he wrote the seminal article “A syndrome produced by diverse nocuous agents”, as a brief letter published in *Nature* in which he described signs that he called “general alarm reactions of the organism” such as gastric ulcers, thymicolymphatic involutions and morphological changes in the adrenal glands. He interpreted these changes as a non-specific adaptive response to various kinds of agents. Notably, he did not use the word “stress” in this first article, but his first comprehensive monography published in 1950, was simply titled “Stress” (Figure 1.1), and has caused debate ever since.

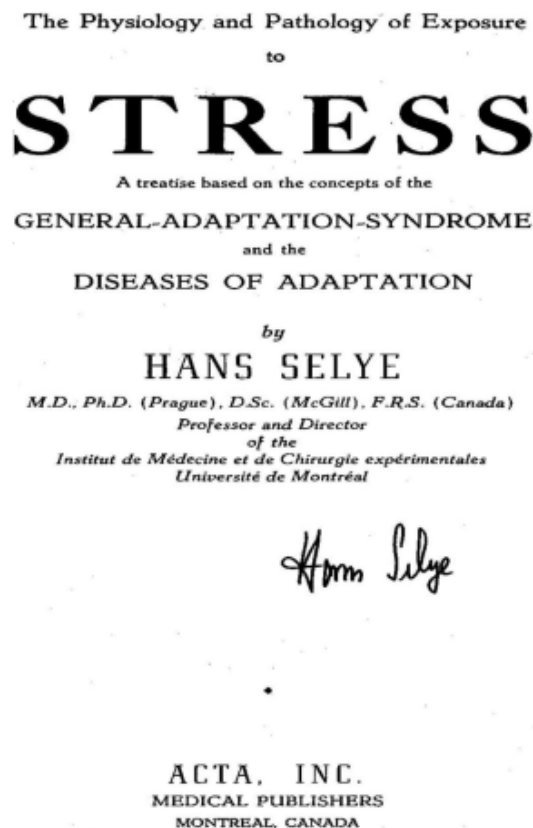


Figure 1.1: The first monograph published by Hans Selye in 1950 (Selye, 1950).

In 1950, his article in the *British Medical Journal* entitled “Stress and the gen-

eral adaptation syndrome”, described initial observations about his hypothesis that stimuli that challenge an organism, either physically (e.g. infectious agents) and psychologically (e.g. exposure to fearful stimuli), can increase the risk of illness and even death.

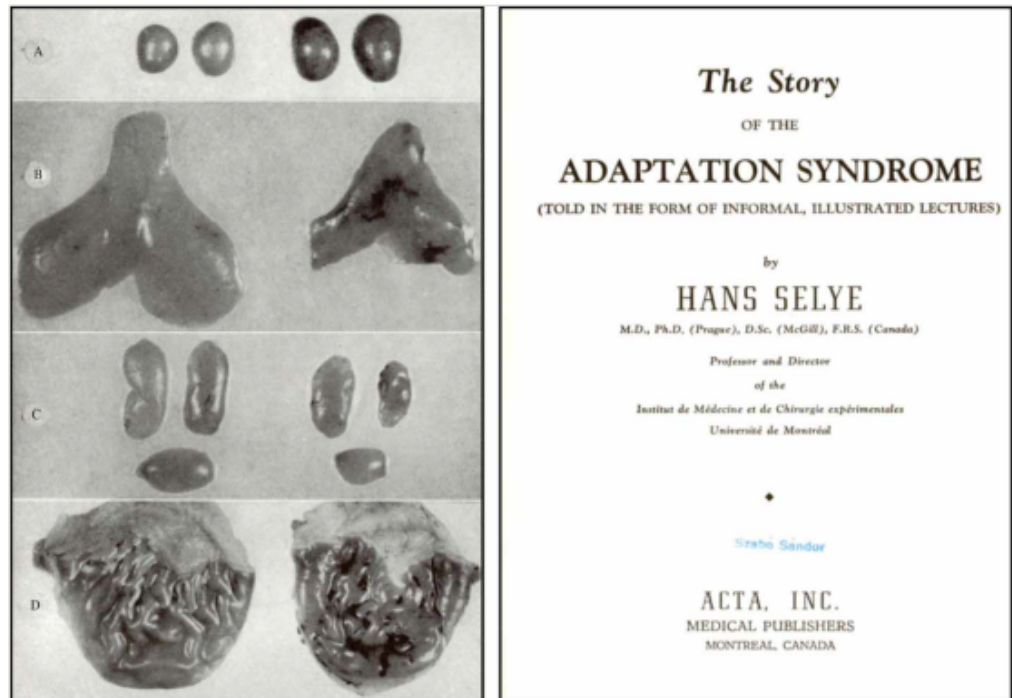


Figure 1.2: The story of the Adaptation Syndrome, by Hans Selye 1952.

Left panel: pictures from the publication. From top to bottom: adrenals, thymus, iliac lymph nodes, and gastric mucosa of a normal rat (left) and the same organs of a rat immobilized on a metal board for 24h (right). In the distressed rat there is evident enlargement of the adrenals, atrophy of the thymus and lymph nodes and gastric erosions. Right panel: Cover to the text (Selye, 1952).

He hypothesized that stress acted through a mechanism that he called the “general adaptation system”, which caused both defense and damage. The defense modulated the responses to injuries, but stress also caused some degree of damage. He concluded that the different stressors can provoke similar consequences in different individuals and one of the main effects was the enlargement of the adrenal glands (see Figure 1.2).

Before Hans Selye another scientist, Walter B. Cannon, investigated the neurochemical and neuroanatomical implications of stress responses and investigated the role of

catecholamines in the “fight and flight” reactions as an emergency response to threat in laboratory animals (Brown and Fee, 2002; Cannon, 1936). Selye was therefore not the only early researcher to investigate stress responses and the adrenal glands, but he was the first to suggest that not only catecholamines produced by the adrenal medulla but also steroids produced by the adrenal cortex, under the influence of adrenocorticotrophic hormone (ACTH) and hypothalamic releasing factors/hormones play a role in the stress reaction.

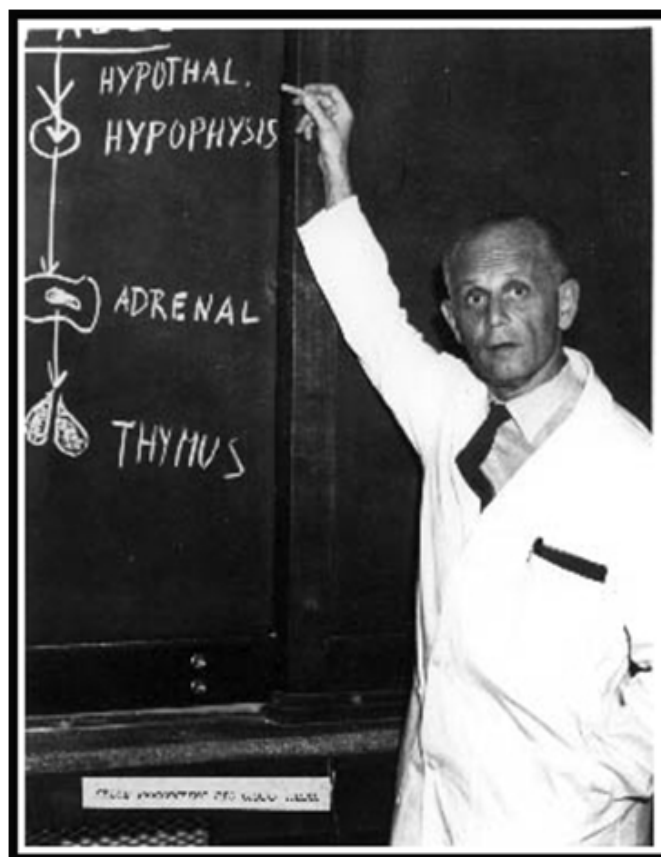


Figure 1.3: Hans Selye (Szabo *et al.*, 2012).

From then on, the role of the Hypothalamic-Pituitary-Adrenal (HPA) axis system, also called the endocrine stress system, was considered of paramount importance in the regulation of mammal behaviour, and the role of adrenocortical hormones, along with the other components of the stress system, has been recognized as crucial to the organism’s ability to cope with threats and successfully adapt during its life. Selye’s papers now have more historical than scientific value but remain highly influential, especially in the popular understanding of “stress”. A large volume of research

in the last 75+ years has shown that neurological, cardiovascular and endocrine mechanisms are involved in complex and integrated stress mechanisms and responses; with stress hormone receptors found in areas of the brain that are crucial for both emotional and cognitive activities.

1.3 HPA, Stress, Corticosteroids And Behaviour

The principal components of the stress response consist of the hypothalamic-pituitary-adrenal (HPA) system (most commonly known as the HPA axis), the locus coeruleus-norepinephrine (LC-NE) system, and the extrahypothalamic corticotropin releasing hormone (CRH) system.

The locus coeruleus (LC) is a small, compact group of norepinephrine (NE) concentrating cells located in the pons, which send projections to every distinct functional region of the neocortex, thalamus, limbic system and hypothalamus, as well as the brainstem nuclei and the spinal cord. Stressful events stimulate the HPA axis and the LC-NE system, which results in a wide range of physiological, endocrine and behavioural effects.

The HPA axis is essential for the control of neural, endocrine and immune responses to challenges. Hypothalamic secretions constantly control the pituitary gland via biochemical feedback messages from the periphery. Corticotrophin releasing hormone/factor (CRH/CRF) and another important hormone, arginine vasopressin (AVP) are secreted by neurons in the hypothalamus.

These hypothalamic factors modulate corticotroph cells in the pituitary gland and increase the synthesis and secretion of adrenocorticotrophic hormone (ACTH).

Both CRH and CRH receptors are important for the secretion of ACTH under stressful conditions whose basal level regulation depends on AVP and its receptors (Gibbs, 1986; Zimmermann *et al.*, 2004). When an animal is stimulated by a stressor, CRH stimulates the production of ACTH in the pituitary: this hormone induces the release of corticosteroids from the adrenal glands. Corticosteroids are a class of steroid hormones secreted from adrenal cortex that are deeply involved in the regulation of energy, immune responses and stress responses. The main corticosteroid in humans is cortisol, while in other species as rats and birds, the main corticosteroid is corticosterone (Carlson, 2010). As mentioned above, stress response can be elicited by a negative threat or a positive challenge. Prolactin and growth hormone are also secreted in the

pituitary in response to stress. Cortisol, prolactin and growth hormone influence the immune system, and have been used to assess the level of stress in animals in experimental situations (Cuatrecasas Cambra, 2009).

Corticosteroids and catecholamines inhibit the production of proinflammatory cytokines and stimulate the production of antiinflammatory cytokines and stress conditions may suppress or potentiate cytokine actions by potentially disrupting the balance between pro and anti-inflammatory action of these substances (Elenkov and Chrousos, 2002). In turn, cytokines act directly on pituitary cells as well as indirectly by controlling the release of hypothalamic factors (Miller *et al.*, 2002; Murali *et al.*, 2006; Zunszain *et al.*, 2011).

The HPA axis and the locus coeruleus-CNS network are not independent of one another because activation of one system tends to activate the other. For example, CRH activates the Locus Coeruleus in addition to its primary function of stimulating ACTH release (Curtis *et al.*, 1999; Jedema and Grace, 2004; Valentino *et al.*, 1983). HPA and the sympathetic nervous system are stimulated in stress conditions and a population of CRH parvocellular neurons project to extra-hypothalamic sites, including limbic nuclei, which are important for emotional responses, as well as the brainstem.

Corticosteroid hormones secreted as a consequence of stress also regulate the expression of an important inhibitory neurotransmitter, the gamma-amino-butyrric acid (GABA) (Orchinik *et al.*, 2001). Changes in GABA receptors have also been demonstrated following administration of exogenous corticosteroids.

Acute and chronic stress have been shown to alter the expression of GABA and a reciprocal regulation of stress hormones and GABA receptors has been postulated (Mody and Maguire, 2011).

An increasing amount of evidence show that the stress-induced release of corticosteroids, induces changes in glutamate neurotransmission in the hippocampus, prefrontal cortex and amygdala. Glutamate is a major excitatory neurotransmitter in the central nervous system and changes in glutamate transmission can have an important influence on cognitive processes and emotions (Graybeal *et al.*, 2012; Popoli *et al.*, 2011).

Corticosteroid hormones produced by adrenal glands and also exogenous corticosteroids might therefore have an impact on animal brains through very complex interconnections consisting of both direct actions on receptors and neurotransmitters and

feedback systems.

Because the production of corticosteroids, stimulated by ACTH, are secreted in a pulsatile fashion with peaks that occur after 15-30 minutes of an ACTH pulse with variations during the day that follow a circadian rhythm, assessment of corticosteroid levels and interpretation of their action is complex (Kolezvská *et al.*, 2003; Leal and Moreira, 1997; Miller and Gronfier, 2006). This is complicated further by the finding that there are refractory periods when the HPA is not sensitive to activation by mild stressors (Young *et al.*, 2004).

For example, Windle *et al.* showed that exposure to noise stress in female rats evoked a significantly smaller response when stress exposure coincided with a non secretory phase of pulse (Windle *et al.*, 1998).

Corticosteroids exert negative feedback control over ACTH secretion with both a rapid and a delayed feedback action. The rapid feedback inhibits the release and synthesis of ACTH in the hypothalamus immediately after a rise in circulating corticosteroids, while the delayed feedback acts via epigenomic action on glucocorticoid receptors in the brain. The epigenomic action of corticosteroids alters transcription of target genes and leads to a change in protein synthesis. Corticosteroids act on the hypothalamus in particular, with a negative feedback action that prevents a continued activation of the HPA axis (Gómez *et al.*, 1998; Herman *et al.*, 2012), but also the hippocampus. CRH is activated after exposure to stress, but it also acts as a neurotransmitter in numerous brain regions.

Several studies have demonstrated that peptide hormones like CRH have functional targets in the brain that are unrelated to the neuroendocrine HPA circuit. However, both classes of function of CRH are activated by factors that disturb organism homeostasis (Amodio and Harmon-Jones, 2011; Claes, 2004; Kovács, 2013; Majzoub, 2006).

1.3.1 Stress does not have the same consequences in all individuals

Despite the description of a general adaptation syndrome, it is important to recognise that stressful events and, as a consequence, the action of stress hormones exert their effect on individuals who are different from each other. Every individual can encounter stressful experiences but not every individual experiences the same con-

sequences. There are individuals that are more resilient to stress and environmental, genetic, epigenetic and neural mechanisms that underlie resilience are involved in the individual differences in stress responses (Hughes, 2012). Resilience seems to be mediated by adaptive changes in several neural circuits involving numerous neurotransmitter and molecular pathways (Degnan and Fox, 2007; Feder *et al.*, 2009). Environmental influences during the developmental period affect the neural and hormonal pathways controlling emotional responses and behaviour. The developmental period is therefore crucial because, as a consequence of external stimulation, the neuroendocrine system and the cascade of hormonal and neurotransmitter responses are shaped and the “tone” of the stress processing system is adjusted. In primate and rodent models it has been shown that early life stress provoked by deprivation of maternal care and early physical interactions with peers are important risk factors for aggressive behaviour in adulthood (Veenema, 2009). Genes and environment control of neuroendocrine mechanisms are therefore part of the basis of individual vulnerability to stress.

1.3.2 Stressors: what are they?

Stress stimuli, also called stressors, can be physiological or psychological, the former involving physical challenges, the latter a reaction to an aversive stimulus without direct physical modification of the animal’s body.

Physiological stressors challenge body integrity and physiological balance, a balance that has to be maintained around a relatively narrow range of parameters such as, for example, a range of temperatures, a range of extracellular sodium concentrations or a range of blood glucose levels. Any challenge to these balances is taken under homeostatic control. Homeostasis is a condition of stability of an organism that should be reached through the responses of the stress system to challenges (Cannon, 1929; Chrousos and Gold, 1992).

Psychological stressors, are processed through higher order brain circuits and involve learned, emotional and cognitive processes. Like physiological stressors, psychological stimuli such as, for example, social conflicts, inappropriate handling, fearful stimuli, perception of an inability to cope and many others also activate parvocellular neurosecretory motor neurons in PVN. Visceral responses to psychological stressors also include cardiovascular, respiratory, gastrointestinal and thermoregulatory changes and are stressor specific. Although it may seem that psychological and physical stressors

activate different pathways at a functional neuroanatomical level, stressors are often compound-both psychological and physical (Kovács, 2013). For example, an analysis of medical records of USA veterans returned from Iraq showed a significantly higher frequency of combination of physical problems and post traumatic stress disorders compared with the frequency in which physical and psychological conditions were present in isolation (Lew *et al.*, 2009), while eustress seemed to be associated with improvement of physical health (Edwards and Cooper, 1988).

1.3.3 Corticosteroids

Corticosteroids and catecholamines are still recognised as the main players in stress responses. Catecholamines play important roles in modulating impulsivity, arousal and attention; while corticosteroid effects are more indirect. Corticosteroids effects are mediated by mineralcorticoid receptors (MRs) and glucocorticoid receptors (GRs) in brain areas that are crucial for memory, learning and emotions. MRs have an affinity for corticosteroids that is 10-fold higher than GRs (De Kloet *et al.*, 1998; Zimmer and Spencer, 2014), which means corticosteroids preferentially bind to them ahead of the GRs in the body. MRs are mainly expressed in the hippocampus, a brain region that is crucial for memory processes. Under baseline conditions they are largely occupied, but during stressful times they become saturated, so the occupation of GRs increases. GRs are distributed in different areas in the brain but are more abundant in hypothalamic CRH neurons and the pituitary. When MRs are predominantly activated hippocampal neurons receive excitatory activation, while additional activation of GRs induces an impairment in hippocampal transmission. In summary, MRs are largely saturated by basal level of hormones, but GRs are activated by high corticosteroid levels such as in stressful conditions. This means that when corticosteroid levels are low (and MRs are not sufficiently activated) or too high (GRs become predominantly activated), memory and learning may be impaired (Gómez *et al.*, 1998; Prickaerts and Steckler, 2005). When, for example, environmental stimuli induce fearful arousal, high levels of circulating corticosteroids saturate GRs and cause related behavioural responses. Such responses increase survival in the natural environment but chronic activation of stress responses might lead to more problematic states in the short-term such as anxiety, phobias and depression. Like endogenous corticosteroids, exogenous corticosteroids bind to GRs and MRs although with different potencies. Corticosteroid drugs are used for their glucocorticoid action and their mineralcorticoid action

varies depending on the type of drug. Cortisol is the standard comparison for the glucocorticoid and mineralcorticoid potencies and, for example, dexamethasone has a glucocorticoid potency that is 25-80 times compared with cortisol but has no mineralcorticoid potency, while prednisolone has 4 times glucocorticoid potency and 0.8 times mineralcorticoid potency compared with cortisol (Rhen and Cidlowski, 2005). The amygdala is a key structure in the fear system and corticosteroid levels affect the amygdala and a variety of other structures, influencing emotional processes. The hippocampus, amygdala and prefrontal cortex are modified by stress and, as a consequence, affective states and behavioural responses can change (Pêgo *et al.*, 2008). The mediator of stress responses can have potentially protecting or damaging effects. Physiological stress responses are indispensable for dealing with life challenges and should be followed by the organism returning to a state of stability, of homeostasis. The involvement of the hippocampus can potentially be detrimental in many ways. For example memory function can be impaired by high levels of cortisol in aged human patients (Lupien *et al.*, 2009) and both chronic stress and corticosterone treatment have been shown to cause impairment of hippocampal-dependent memory tasks in laboratory rats (Clark *et al.*, 2000; Diamond and Rose, 1994; Endo *et al.*, 1996; McLay *et al.*, 1998; Park *et al.*, 2008). The onset of structural changes in the hippocampus as a consequence of the action of stress hormones has been investigated in many studies, with CA3 pyramidal cells in the dentate gyrus being shown to be particularly vulnerable to stress. Neurogenesis and cell survival in the dentate gyrus can be suppressed in stressful conditions (Gould and Tanapat, 1999) and the dendrites of CA3 cells can show stress-induced modifications (Joëls, 2007; Stewart *et al.*, 2005). In summary, adrenal steroids seem to be important mediators of the changes in hippocampal neurons during stress. There are also multiple interactions with other neurochemical systems include serotonin, endogenous opioids, GABA receptors, the calcium channel currents and glutamate (McEwen and Magariños, 2001). For example, it has been suggested that chronic restraint stress increases the release of glutamate in rats (Magariños *et al.*, 1997). Glutamate has a critical role in controlling synaptic excitability and its dysfunction can cause behaviours of concern (Aida *et al.*, 2015).

Furthermore, chronic restraint stress can cause alterations in prefrontal cortical dendritic morphology and these changes have been related to attention impairments. The prefrontal cortex (PFC) is involved in extinction, a learning process that is very im-

portant for adaptation, and chronic stress has been related to impairment of extinction of a fear conditioned task in rats. The medial PFC is a target for corticosteroids involved in stress response and stress can cause, as mentioned above, an increase in glutamate, along with acetylcholine release. These neurochemical changes due to chronic stress cause atypical changes in the dendrites of pyramidal neurons of PFC and the consequences are functional changes and, eventually, behavioural changes. For example, the retrieval of the memory of fear extinction was impaired in distressed rats: i.e. the animals did not recall the extinction previously learned (Miracle *et al.* 2006). Chronic restraint stress was also reported to enhance amygdala-dependent fear conditioning and unlearned fear (Conrad *et al.*, 1999). The observed increase in aggression between rats in the same cage as a consequence of chronic stress has also been considered to be a sign of amygdala hyperactivity (Wood *et al.*, 2003), and chronic corticosterone treatment in mice produced anxiogenic effects that could be related to amygdala activity (Ardayfio and Kim, 2006; Makino *et al.*, 1994).

It therefore appears that whilst corticosteroids are essential for protecting against adverse events, their effects can turn from adaptive to maladaptive in cases of imbalanced actions that last for prolonged periods of time (De Kloet *et al.*, 1999). Intensive, prolonged and repeated stimulation of the stress system can lead to the onset of anxiety, with a range of maladaptive behaviours such as an increase in avoidance strategies, aggression, displacement activities and stereotypies. The boundary between an adaptive stress response and a maladaptive one is not easy to define when considering dog behaviour, and some behaviours that owners perceive as problems might be just normal coping strategies, part of the normal range of an animal's behaviour. The attempt of an animal to perform adaptive behaviours in contexts where adaptation is difficult or impossible might lead to maladaptive, non functional behaviours (Mills, 2003). What often makes the difference between normal and problematic behaviours is the intensity and duration of behaviours, often with the inclusion of signs of anxiety. These signs overlap with more general signs of acute and chronic distress, with stress and the stress response often at the heart of the issues. A qualitative, holistic evaluation of dog behaviour is probably more applicable and sufficiently reliable, being the evaluation of "the whole animal" a useful approach to detect signs of stress and anxiety and infer emotional states (Mills *et al.*, 2006; Wemelsfelder, 1997). Because stress hormones affect the animals' brain, emotion and cognition, being able to distinguish between normal and problematic behaviours is important in order to be able

to recognize their effects.

1.4 Behavioural And Psychological Side Effects Of Corticosteroids

Many studies have shown how endogenous and exogenous corticosteroids might affect mammal brains in different ways. They can modulate neuron excitability through different mechanisms and neurotransmitters, and can cause changes in the prefrontal cortex and limbic system, structures that are profoundly interconnected. The prefrontal cortex has been described as an important area for cognition, while the limbic system is deeply involved in emotions. The prefrontal cortex mediates the cognitive regulation of emotion, the amygdala exerts emotional influences on cognitive processes and the hippocampus is greatly involved in memory formation. It has been shown that both acute stress and exogenous corticosteroid exposure might enhance the memory of emotional experiences and impair memory abilities (Barsegyan *et al.*, 2010; Salzman and Fusi, 2010).

Explicit short term memory also called working memory, which is the ability to retain information and then use it to guide future behaviours, is an important function of the prefrontal cortex and in experimental treatment with the exogenous corticosteroid dexamethasone spatial working memory and cognitive flexibility were impaired in rats. Furthermore, memory retrieval was shown to be impaired by both stressful events and administration of the endogenous corticosteroid corticosterone (Cerqueira *et al.*, 2005; Schutsky *et al.*, 2011).

An increase in anxiogenic-like behaviour has also been observed in mice after chronic administration of corticosterone (Ardayfio and Kim, 2006). Glucocorticoid administration before an experiment involving fear conditioning in rats increased the acquisition of the memory of the fearful event (Thompson *et al.*, 2004), but rats chronically treated with corticosteroids needed more time and trials to accomplish a maze learning task compared with controls. The authors concluded that chronic administration of corticosteroids impairs learning and memory (Endo *et al.*, 1996).

Ramos-Remus *et al.* investigated the effects of prednisolone (a drug commonly used for long term management of inflammatory conditions in veterinary practice) administration in rats using a water maze to assess disturbance in learning and memory, and also investigated neural and glia cell changes. Rats treated with prednisolone

showed a significant delay in learning and memory retention of the maze task compared with controls and the degeneration index of neural cells was significantly higher compared with control animals. They concluded that prednisolone produces learning and memory impairment and changes in neurons and glia cells in cerebral regions involved in learning and memory (Ramos-Remus *et al.*, 2002). These results and the use of exogenous corticosteroids for treating neural disorders seem to be in contrast because corticosteroids are also used for their neuro-gliomodulation effects (Gonzalez-perez *et al.*, 2010). Corticosteroids exert several important effects by targeting glia cells and the most abundant glia cells in the brain, astrocytes, play important roles in neurotransmitter reuptake and release, modulation of synaptic transmission and hormonal signaling. Synthetic corticosteroids modulate neural cells and their actions on glia and neurons have been shown to produce detrimental or positive effects, so while in rats in the study of Ramos-Remus *et al.* (Ramos-Remus *et al.*, 2002), prednisolone appeared to induce degeneration of neural cells, the same corticosteroid drug did prevent the reduction of brain-derived neurotrophic factor (BDNF) and neural growth factor (NGF) mRNA expression in the brain in experimental autoimmune encephalomyelitis in mice, although in combination with another drug (Chen *et al.*, 2009). The different effects of exogenous corticosteroids on neural cells have not been completely clarified, and appear to be mediated by dose, individual features, and brain region phenomena (Numakawa *et al.*, 2012). A reliable picture of possible behavioural effects of corticosteroids might be reached by merging the evidence of the available and future studies that investigate corticosteroid effects at different levels, from cell responses to the whole animal behaviour in different contexts. Rats that received corticosterone before social encounters were found to be more aggressive compared with non-treated controls, with corticosteroids increasing aggression in social challenging situations, but not in normal conditions (Mikics *et al.*, 2007, 2004). Such experiments in laboratory animals provide evidence that not only stressful events but also the administration of exogenous corticosteroids can influence emotion, cognition and behaviour.

Corticosteroid drugs are widely used in veterinary medicine as in modern human medicine. Although in human medicine the behavioural and psychological side effects of these drugs have been reported since they started being used, and these drugs have been shown to induce clinically significant and sometimes severe psychological, cognitive, and behavioural disturbances (Brown *et al.*, 1999; Kenna *et al.*, 2011; Patten

and Neutel, 2000), virtually no literature was available on these kinds of side effects in dogs before the publication of our studies from this thesis (Notari and Mills, 2011; Notari *et al.*, 2015). It is therefore important to appreciate what these effects in humans might be and how the equivalent problem might manifest in companion dogs. Descriptions of the psychiatric side effects of corticosteroids in humans date back to the 1950's, when these drugs started being prescribed on a regular basis. For example, in 1951 Borman and Schmallerberg, in the Journal of American Medical Association, reported a case of the suicide of a patient in treatment with corticosteroids (Borman and Schmallerberg, 1951). From then on, several case reports concerning psychiatric side effects of corticosteroids have been published. Selwin Brody in 1952 described several clinical cases, reporting psychiatric observations in patients that had received treatment with corticosteroids. He described some emotional reactions to cortisone and ACTH as euphoria, psychosis, ambivalent behaviours and depression (Brody, 1952).

Many other cases have been reported in the following decades and, because of the wide scale use of this medication in human medicine, with its frequent prescription for long term treatment, investigation of this topic has received a lot of attention (Kenna *et al.*, 2011; Judd, 2014). Individual cases or case-series reports have illustrated impressive severe neuropsychiatric outcomes in adults, adolescents and children (Brown and Chandler, 2001; Brown *et al.*, 1999; Drozdowicz and Bostwick, 2015; Mian *et al.*, 2007; Warrington and Bostwick, 2006). It was reported that 6% of patients who received corticosteroids had severe psychiatric side effects and that corticosteroid dose was a risk factor for these kinds of side effects, with a reduction of the dose resulting in recovery from psychiatric symptoms (Dubovsky *et al.*, 2012). The case-report literature concerning psychiatric side effects of corticosteroid drugs often lacks scientific validation. In addition the implication of other influencing factors such as the decrease in the quality of life due to pain and disease, the onset of other physical side effects of these drugs as increased appetite and change in body shape that might have influenced both the individual and social life of patients (Bolton *et al.*, 2010), along with the bias inherent in patient or care-giver reports suggest caution when considering this evidence (Stella *et al.*, 2015). Nevertheless, as far as the development of psychiatric symptoms is concerned, the extensive reports of these side effects along with a few controlled studies in human patients suggest that the issue is an important one.

An example of a more structured, cross sectional and longitudinal study concerning psychological side effects of corticosteroids in humans is the study of Keenan *et al.* (Keenan *et al.*, 1996). One of the investigated effect of corticosteroid drugs in human beings is their effects on memory and it was shown that treated patients performed worse in explicit memory tasks but not in implicit memory tasks, compared with controls. Explicit memory is the conscious recall of information while implicit memory is “remembering without recall”, a sort of unconscious memory like being able to perform previously learned task without the need to think about how to perform it, for example riding a bike or dancing. The longitudinal part of this study was conducted following the patients across 3 months of therapy and it was shown that both acute and chronic administration of corticosteroids can negatively affect memory (Keenan *et al.*, 1996). Other studies have suggested that the age of human patients appears to be unrelated to the risk of psychiatric symptoms during corticosteroid treatment, while a slightly higher prevalence may be found in women, but this kind of prevalence bias might be related to the fact that many diseases that require corticosteroid treatments are more frequent in female patients (Cerullo, 2006).

Some reports in human patients suggest that corticosteroids might play a role in the onset or worsening of bipolar disorders, psychiatric disorders that cause exaggerated and abnormal shifts in mood with important influence on the ability to carry out normal daily tasks (Panwar and Lassi, 2011; Pies, 1981).

The diagnosis of what is called “steroid psychosis” is a challenging one for human psychiatrists. Mood disorders are frequently described as the most evident aspect of steroid psychosis along with corticosteroid-induced mania, but these disorders are not easy to distinguish from primary disorders that might be worsened during corticosteroid treatment (Cerullo, 2006). So, investigation of the effects in psychiatric patients is inherently more challenging.

Cognitive impairment, in terms of memory impairment and even dementia, due to corticosteroids has also been described (Martignoni *et al.*, 1992; Varney, 1997). For instance, Sacks and Shulman reported the case of an elderly patient who was treated with prednisolone and developed a severe dementia, some of whose symptoms resolved after discontinuation of the drugs, but signs of memory and attention deficits were still present six months after the discontinuation of the therapy (Sacks and Shulman, 2005). But cognitive and attentional effects are not limited to the elderly; Wolkowitz *et al.* describe the case of a 10 year old child who received different kinds of

corticosteroids including prednisolone (oral administration, 60 mg/day), methylprednisolone (endovenous administration 80-120 mg/day) and dexamethasone in addition to inhaled medications to treat asthma and, after the beginning of the corticosteroid treatment, he started to show signs of depression, irritability, memory and attention problems and also avoidance of social contact (Wolkowitz *et al.*, 2007). After discontinuation of the corticosteroid therapy these psychiatric signs gradually improved but brain Magnetic Resonance Imaging performed after 3 years revealed that the patient's hippocampal volume was 19.5% smaller than that of the young patient's twin. This might suggest a significant developmental impact on the immature individual, consistent with that reported in laboratory animals (Edwards and Burnham, 2001). A survey conducted in 2012, quantified the impact of corticosteroid psychiatric side effects in humans by analysing the UK electronic medical database called "The Health Improvement Network (THIN)". That has the longitudinal medical records including all prescriptions of patients included in the database, from 1990 to 2008. The authors identified prescriptions for oral corticosteroids and compared the incidence of neuropsychiatric disorders in patients exposed and non-exposed to corticosteroid therapies. They assessed hazard ratios associated with the first prescription issued for corticosteroid drugs and performed a sensitive analysis that excluded prescriptions for psychotropic drugs, used as a definition of previous neuropsychiatric disorders. They also selected a mix of first and later exposure to corticosteroids in order to be able to understand whether previous exposure to corticosteroids was associated with a higher risk of developing a psychiatric disorder. They found that, compared with a patient population unexposed to corticosteroid therapies, exposed patients showed a five to seven fold increased risk of suicide and an even greater risk for delirium, confusion, disorientation and mania. An increased risk was also found for depression and panic disorder in exposed patients.

Furthermore, high doses of corticosteroids and a prior history of neuropsychiatric disorder were associated with higher risk of negative psychiatric outcomes. On the contrary, a prior history of corticosteroid treatments was associated with a lower risk. This latter outcome might be explained by the decreased probability of receiving a second prescription for corticosteroids in patients that had psychiatric adverse events the first time (Fardet *et al.*, 2012).

In patients with a history of multiple treatment courses with corticosteroids larger doses of corticosteroids and a previous history of neuropsychiatric disorder associated

with corticosteroid therapy was associated with the risk of having the same disorder in subsequent corticosteroid treatments. Only 1.3% of severe psychiatric symptoms were reported in patients with daily doses of prednisone equivalents lower than 40 mg, while 18.4% of severe psychiatric symptoms were reported in patients that received more than 80 mg of prednisone equivalent every day. The overall risk of developing neuropsychiatric adverse events in patients exposed to corticosteroid treatments was 15.7 in every 100 patients and was 22.2 percent in patients on their first treatment with corticosteroids.

1.5 Conclusion

Given these findings and the general recognition that stress is an important risk factor for behaviour problems in dogs, together with the fact that corticosteroids are widely used in veterinary practice, it is important to consider that exogenous corticosteroids might affect the cognition and behaviour of companion dogs. This research represents the first attempt to discover what kinds of changes can be expected in such dogs when these drugs are prescribed.

Chapter 2

The Effects Of Exogenous Corticosteroids On Dog Behaviour: First Exploratory Survey

In this chapter a first exploratory survey about possible behavioural effects of corticosteroid drugs in dogs is described. This survey was informed by the available literature about behavioural and psychological side effects of corticosteroid drugs in other species, including human beings. The main goal was to generate items for future use in a controlled structured questionnaire. The perceptions of behavioural changes in dogs during corticosteroid therapy were investigated through semi-structured open interviews of the owners of 31 dogs of different breeds, genders, and ages and eleven owners reported behavioural changes in their dog behaviours.

2.1 Introduction

In chapter 1 it has been suggested how, in other species, corticosteroids might have remarkable behavioural side effects. In veterinary medicine, although the physical side effects of corticosteroids have been widely described, reports of psychological or behavioural side effects are anecdotal (Dodman and Shuster, 1998), and so far there are no literature data about the incidence of these kinds of problems in pet animals. In 2008 Klinck *et al.* investigated about possible association between pruritic skin disease and aggression, fear or anxiety-related behaviours in dogs (Klinck *et al.*, 2008). The results of their survey showed a significantly increased reactivity to noises and thunderstorms in dogs treated with corticosteroids, without any significant effect related with pruritic skin diseases. This survey probably represents the first report about possible behavioural effects of corticosteroids in dogs and it is therefore particularly interesting from our point of view because it shows that, at least in the owners' opinion, one of the main sign of dermatological conditions and probably the most evident, pruritus, was not considered as significantly associated with behavioural changes, while treatment with corticosteroids seemed to influence dog behaviour. One of the most widespread use of corticosteroids in veterinary medicine is in dermatology, where these drugs are used for their immunosuppressive and anti-inflammatory properties in the treatment of a variety of skin conditions, as for example atopic dermatitis (Olivry *et al.*, 2010). Systemic corticosteroids were shown to be prescribed in 20% of skin cases in primary veterinary care practice (Hill *et al.*, 2006). Corticosteroids are widely used in veterinary medicine for a number of conditions, not just in dermatology, and they are among the most prescribed drugs for pet animals (Sousa, 2009;

McDonald and Langston, 1995). According to O'Neill *et al.*, the use of corticosteroid therapies in veterinary practice is quite widespread, with 14.55% of dog consultations in primary care practice receiving systemic corticosteroid prescriptions (O'Neill *et al.*, 2012). Corticosteroids are potent anti-inflammatory and immunosuppressive agents and the majority of therapeutic applications for these drugs fall into these classifications. Corticosteroids are also prescribed for treating neoplastic diseases, allergic reactions and are also employed in some emergency protocols (Ferguson *et al.*, 2009). For the purpose of this study, treatments for life-threatening or very severe conditions were not considered because the drug effect in those cases would more probably be overcome by the effect of the distress due to pain.

Due to the presence of corticosteroid receptors in almost all cells, both the desired and undesired effects of corticosteroid therapy are manifold, making the need for prudent use particularly important (Behrend and Kemppainen, 1997) and veterinarians are becoming more and more cautious in prescribing these drugs as they become more and more aware of the possible drawbacks of corticosteroid therapies (Ferguson *et al.*, 2009; Levine *et al.*, 2008; Torres *et al.*, 2005). However, behavioural side effects, even when mentioned, are not supported by evidence (Dodman and Shuster, 1998; Pageat, 1998).

Corticosteroid drugs can have both mineralocorticoid and glucocorticoid receptor activity, but are mainly employed for their glucocorticoid effects (De Kloet *et al.*, 1999); exogenous corticosteroids can lead to medical complications in dogs, either directly or indirectly by causing, for example, immunosuppression or masking signs that might be important for the diagnosis or monitoring of a disease. The effects of corticosteroids on the brain are complex but result in a cascade of neurotransmitters involved in a range of cognitive processes and behavioural responses (Wolkowitz *et al.*, 2009, see also Chapter 1).

Considering the wide use of corticosteroids in veterinary medicine, it is perhaps surprising that there is this lack of data and awareness relating to potential behavioural side effects, especially since the role of stress hormones in the onset of behaviour problems is widely recognised in the veterinary literature (Overall, 1997; Pageat, 1998). Such information would not only be beneficial to practicing veterinarians, but data about the incidence of behavioural side effects in dogs might also be useful for owners, in order to prevent them inadvertently increasing personal risk to themselves or others when their dogs are under treatment with these drugs.

In order to systematically, investigate this issue, perhaps the first question that needs to be answered is: what kind of behaviours, if any, might owner's observe as a consequence of the administration of exogenous corticosteroids? Using a similar approach to that used in the investigation of behavioural changes associated with chronic pain in dogs (Wiseman *et al.*, 2001), it was hypothesized that owners, through direct observations of their dogs' behaviour during corticosteroid therapy, might provide useful information in order to set a starting point for the development of the present research project.

In this chapter, the first stage of our investigation into the behavioural side effects of exogenous corticosteroids in dogs is reported, in which we aimed to establish the possible behavioural side effects using a systematic owner interview procedure.

2.2 Materials and Methods

Subjects were a self-selected convenience sample of dog owners recruited from clients of veterinary clinics in the north of Italy. Inclusion criteria were that dogs had received therapy with systemic corticosteroids for at least two weeks in the last six months. Semi-structured interviews were conducted, in which owners were asked if they had noticed any change in their dogs' behaviour during the time they were giving corticosteroids. At the beginning of the interview; owners were free to answer without any prompts, then they were prompted with questions about areas of behaviour in which, on the basis of the literature, changes might be expected. Table 2.1 lists the investigated domains. The interviews terminated when data redundancy occurred. The point of data redundancy was determined as the point at which owner interviews failed to generate new information for 10 successive interviews (Sandelowski, 1995; Strauss and Corbin, 1998).

Using this method, the owners of 31 mixed-breed dogs were recruited, 19 male dogs and 12 females of different ages (range of ages from 1 to 13) were included in this preliminary survey. Eighteen dogs had been oral administered methylprednisolone (dose range 0.2-1mg/kg), eight dogs received oral prednisolone (dose range 0.2-1mg/kg), five dogs received dexamethasone via intramuscular injection (dose range 0.01-0.3mg/kg). Further details concerning individual subjects and the dosing procedure used are given in Table 2.1. Nine dogs were also receiving antibiotic therapy. Twenty four dogs suffered from a dermatological condition, five dogs suffered from arthritis, one

dog suffered from myasthenia gravis and one suffered from recurrent otitis (Table 2.2).

QUESTION	INVESTIGATED DOMAINS	QUESTION EXAMPLES
1	General question	“Did you notice any change in your dog’s behaviour during corticosteroid therapy?” “Can you describe this change?”
2	Dog personality	“How would you describe your dog’s personality? Is this changed in any way during corticosteroid therapy?” “Can you describe this change?”
3	Behaviour with family members	“Have you noticed any change towards family members when the dog was under corticosteroid therapy?” “Can you describe this change?”
4	Behaviour with strangers at home and outside	“Have you noticed any change towards strangers or guests at home or people outside, when the dog was under corticosteroid therapy?” “Can you describe this change?”
5	Behaviour when left alone	“Have you noticed any change in your dog’s behaviour when he/she was left alone at home, during corticosteroid therapy?” “Can you describe this change?”

Table 2.1: Continued on next page

QUESTION	INVESTIGATED DOMAINS	QUESTION EXAMPLES
6	Behaviour during walks	<p>“Have you noticed any change in your dog’s behaviour during walks when the dog was under corticosteroid therapy?”</p> <p>“Can you describe this change?”</p>
7	Fears and avoidance behaviours	<p>“Have you noticed any change as far as fearfulness or avoidance of people, animals or situations when the dog was under corticosteroid therapy?”</p> <p>“Can you describe this change?”</p>
8	Barking	<p>“Have you noticed any change in the intensity of barking in general, when the dog was under corticosteroid therapy? Can you describe this change?”</p>
9	Eating	<p>“Have you noticed any change in behaviour around food when the dog was under corticosteroid therapy?”</p> <p>“Can you describe this change?”</p>
10	Drinking	<p>“Have you noticed any change related drinking when the dog was under corticosteroid therapy? Can you describe this change?”</p>
11	Sleeping	<p>“Have you noticed any change in the way your dog sleeps when the dog was under corticosteroid therapy?”</p> <p>“Can you describe this change?”</p>

Table 2.1: Continued on next page

QUESTION	INVESTIGATED DOMAINS	QUESTION EXAMPLES
12	Behaviour with other animals	<p>“Have you noticed any change in behaviour towards other dogs or other animals when the dog was under corticosteroids?”</p> <p>“Can you describe this change?”</p>
13	Behaviour problems	<p>“Have you noticed any behaviour problem that was not present before, when the dog was under corticosteroid therapy?”</p> <p>“Can you describe this change?”</p>

Table 2.1: Areas of dog behaviour that were investigated through open questions. Questions focused on differences on and off corticosteroid therapy.

	Dog Breed/Type	Age (years)	Gender	Medical Condition	Corticosteroid	SD mg/kg	W/MD mg/kg	Concomitant Drug Therapies
1	Charplanina Sheep Dog	5	M	Dermatological	Methylprednisolone	0.6	0.3	Antibiotics
2	Crossbreed	3	F	Dermatological	Methylprednisolone	0.5	0.25	
3	Crossbreed	8	M	Arthritis	Dexamethasone	0.15	0.07	
4	Jack Russel Terrier	2	F	Dermatological	Methylprednisolone	0.5	0.25	
5	Pomeranian	4	M	Dermatological	Methylprednisolone	0.6	0.3	Antibiotics
6	Fox Terrier	6	M	Dermatological	Prednisolone	0.5	0.25	Antibiotics
7	WHWT	9	FS	Dermatological	Prednisolone	0.6	0.3	
8	Crossbreed	3	FS	Dermatological	Methylprednisolone	0.5	0.25	Antibiotics
9	Crossbreed	7	M	Dermatological	Methylprednisolone	1.0	0.5	Antibiotics
10	Beagle	5	M	Dermatological	Prednisolone	0.3	0.3	
11	German Shepherd	2	M	Dermatological	Methylprednisolone	0.4	0.2	
12	Italian Hound	7	FS	Dermatological	Methylprednisolone	0.6	0.3	Antibiotics
13	Crossbreed	3	M	Dermatological	Methylprednisolone	0.5	0.25	

Table 2.2: Continued on next page

	Dog Breed/Type	Age (years)	Gender	Medical Condition	Corticosteroid	SD mg/kg	W/MD mg/kg	Concomitant Drug Therapies
14	Golden Retriever	8	FS	Dermatological	Prednisolone	0.4	0.2	Antibiotics
15	WHWT	13	FS	Dermatological	Methylprednisolone	0.5	0.25	
16	Boxer	1	M	Dermatological	Methylprednisolone	1.0	0.5	
17	Crossbreed	6	M	Dermatological	Prednisolone	0.5	0.25	
18	Miniature Schnauzer	11	F	Arthritis	Dexamethasone	0.3	0.15	Antibiotics
19	Yorkshire Terrier	12	M	Recurrent otitis	Prednisolone	0.4	0.2	
20	Dachshund	3	M	Dermatological	Methylprednisolone	0.4	0.2	Antibiotics
21	German Shepherd	2	F	Dermatological	Methylprednisolone	0.5	0.25	
22	Bull Terrier	2	F	Dermatological	Methylprednisolone	0.6	0.3	
23	Crossbreed	7	M	Dermatological	Methylprednisolone	0.5	0.25	
24	Lagotto Romagnolo	6	M	Myastenia gravis	Prednisolone	0.5	0.25	
25	Golden Retriever	4	M	Dermatological	Methylprednisolone	0.6	0.3	Antibiotics
26	Bernese Mountain	8	F	Arthritis	Dexamethasone	0.2	0.1	
27	German Shepherd	9	M	Arthritis	Dexamethasone	0.1	0.05	

Table 2.2: Continued on next page

	Dog Breed/Type	Age (years)	Gender	Medical Condition	Corticosteroid	SD mg/kg	W/MD mg/kg	Concomitant Drug Therapies
28	Boxer	9	F	Arthritis	Dexamethasone	0.06	0.03	
29	Crossbreed	5	M	Dermatological	Methylprednisolone	0.5	0.25	
30	Maremmano Abruzzese	4	M	Dermatological	Prednisolone	0.4	0.2	
31	Bergamasco Shepherd	8	M	Dermatological	Methylprednisolone	0.8	0.4	

WHWT = West Highland White Terrier; M = male; F = Female; FS = Spayed Female; SD = Starting dose of corticosteroids;

WD/MD = Weaning Dose/Maintenance Dose of Corticosteroid.

Table 2.2: Breed, gender and reproductive status, age, disease, type and doses of administered corticosteroids of the 31 dogs included in the study.

2.3 Results

Eleven owners reported behavioural changes in their dog's behaviour, nine dogs were reported to show more than one behavioural change. Of these nine dogs, two were also receiving treatment with antibiotics (amoxicillin and clavulanic acid). Six dogs reportedly showed nervousness/restlessness, three an increase in startle responses, three food guarding, two a decrease in their activity level, three an increase of avoidance responses, 4 irritable aggression and 2 increased barking. These interpretations were deduced and summarized from the owners' descriptions and were represented by the onset of certain behaviours or by an increase in frequency and/or intensity of them. The results are summarized in Table 2.3.

Dog	Breed	Age	Gender	Medical Condition	Corticosteroid	Concomitant Drug Therapies	Reported Behavioural Changes
1	Charplanina Sheep Dog	5	M	Dermatological	Methylprednisolone	Antibiotics*	Food guarding; irritable aggression
3	Crossbreed	8	M	Arthritic	Dexamethasone	none	Nervousness; increased startle response
7	WHWT	9	F	Dermatological	Prednisolone	none	Nervousness and decreased activity level
10	Beagle	5	M	Dermatological	Prednisolone	none	Nervousness; food guarding
12	Italian Hound	7	F	Dermatological	Methylprednisolone	none	Nervousness; onset /increased avoidance behaviours
15	WHWT	13	F	Dermatological	Methylprednisolone	none	Avoidance behaviours
16	Boxer	1	M	Dermatological	Methylprednisolone	none	Decreased activity level
20	Dachshund	3	M	Dermatological	Methylprednisolone	none	Increased startle response; increased barking
25	Golden Retriever	4	M	Dermatological	Methylprednisolone	Antibiotics*	Food guarding; irritable aggression

Table 2.3: Continued on next page

Dog	Breed	Age	Gender	Medical Condition	Corticosteroid	Concomitant Drug Therapies	Reported Behavioural Changes
29	Crossbreed	5	M	Dermatological	Methylprednisolone	none	Nervousness; increased startle response; increased barking
30	Maremmano Abruzzese/ Shepherd Dog	4	M	Dermatological	Prednisolone	none	Nervousness; irritable aggression

*Amoxicillin and Clavulanic Acid

Table 2.3: Breed, age, gender, medical condition, concomitant therapies and behavioural changes reported in 11 dogs with identified changes during corticosteroid therapies. Dog number corresponds to number in Table 2.2.

Brief case reports on positive cases are given below.

The owner of Dog 1 reported that after a few days of corticosteroid therapy the dog became more aggressive in the presence of food and more aggressive in general when disturbed or approached. The owner of Dog 3 described him as a very sweet and calm dog before the therapy, while after the second injection of dexamethasone (day 3 of therapy) he became restless, very nervous and tended to be startled by even minimal sound. The owner reported that his dog was almost impossible to keep calm, but that after a few days following the interruption of corticosteroid treatment the dog gradually returned to his usual behaviour and reactivity level.

The owner of Dog 7 also reported that after a few days of therapy the dog was more prone to startling at every sound and stimulus but was less active, in general, during the day, while appearing restless in the evening.

Dog 10 received 5 mg of prednisone (0.3 mg/kg) for 2 months and the owner described that after a few days he started to show aggression in the presence of food and became very difficult to manage because he had become very nervous, restless and showed increased attention-seeking by barking and jumping on people. When the therapy was discontinued the dog gradually became more calm and manageable.

The owners of Dogs 12 and 15 both described that their dogs tended to stay isolated from social contexts, in particular when people spoke with loud voices. Dog 12, had been rescued when she was 2 years old and the owner described the dog as fearful and tending to avoid people when first obtained, although this resolved with time. After corticosteroid treatment the owner reported that she seemed to have returned to showing the behaviour she had expressed at the time she was adopted, several years before. Both these dogs reportedly returned to their more usual behaviour when therapy was discontinued.

Dog 16 received 1 mg/kg of methylprednisolone on the first day of therapy. Almost immediately the owner noticed that he became very calm. The owner reported that like many boxers, this dog used to be very lively and jump on people all the time. Under corticosteroid therapy he seemed almost sedated. The dose was reduced by a half (0.5 mg/kg) in the second day of therapy and the dog appeared less sedated but still very calm until the end of the entire period of corticosteroid therapy, when he gradually returned to his previous behaviour.

Dog 20 reportedly became more reactive and started to bark very frequently and intensely at even minor stimuli a few days after the beginning of corticosteroid therapy.

During the second week the owner interrupted the therapy and the dog apparently became gradually calmer and returned to his previous behaviour. The signs of the dog's dermatological condition which preceded and followed the corticosteroid therapy and the behaviour change, had not been solved at the time of interview.

The owner of Dog 25 reported that during the first week of corticosteroid therapy the dog started to growl at anyone who came close to his food bowl and also bit a family person who tried to pet him while he was lying on his bed. The dog had started to suffer from the dermatological problem some days before the onset of the therapy, but did not apparently show any sign of aggression until the initiation of the therapy. When corticosteroids were discontinued the dog stopped growling in similar contexts.

The owners of Dog 29 reported that after a few days of treatment the dog started to become nervous and agitated, and to bark incessantly at almost any stimulus, to the point that they could not leave him in the garden anymore. The dog also started to react fearfully towards people during walks and to bark at them. Although the dermatological problem was not resolved, they decided to interrupt the therapy in the third week of treatment and the dog gradually returned to his previous behaviour.

The owner of Dog 30 reported that, after a few days of therapy, he started to become nervous and showed aggression towards both a stranger and a family member for no apparent reason beyond coming close to him. These episodes happened during the second week of therapy while the dog was taking half of the starting dose of methylprednisolone. The owner decided to interrupt the therapy and to treat the dog just with antibiotics. After the interruption of corticosteroid therapy the dog seemed to return to its previous behaviour but the dermatological condition worsened. After one month the veterinary surgeon suggested starting corticosteroids again, at the dose of 0.3 mg/kg of prednisolone. After a few days the dog began to become nervous again and bit the owner on his arm when he tried to hold him.

2.4 Discussion

The sample used in this first stage of the research might have been biased towards positive reports due to the possible perception that corticosteroid drugs are dangerous, a perception that seems to be quite common among human patients (Charman *et al.*, 2000; Janson and Becker, 1998). However, our findings suggested that the alterations in behavioural repertoire in dogs during corticosteroid therapy deserved

further attention with more rigorous quantitative methodologies, providing valuable information that we could use to proceed towards the next phase of our investigation. The method of collecting data through the reports of carers, in a similar manner to that used here, has been employed in several studies, both in humans and in non-human animals (Hall *et al.*, 2003; Wells and Hepper, 2000). It has been argued that since owners spend considerable time in contact with their animals, they are in the best position to provide interpretive information on the overall patterns of behaviour of their own dogs (Wemelsfelder, 1997; Wiseman *et al.*, 2001). The results are consistent with the predictions that might be made on the basis of the scientific literature described in Chapter 1, which would suggest a general increased reactivity to stimuli, especially potentially aversive ones (De Kloet *et al.*, 1999; Korte, 2001). Nonetheless, it must be recognised that other factors in the study population might be important for the apparent onset of behavioural changes. For example, the stress and discomfort associated with conditions such as pruritus and pain, that necessitate the use of corticosteroid therapies, might alter the behaviour of the animals (Seksell and Lindeman, 2001). If a subject is stressed either physiologically or psychologically, ACTH production is enhanced, regardless of the levels of circulating cortisol, and so it might be expected that the behaviour of dogs who already have some form of behavioural disturbance, may be exacerbated by treatment, rather than the problem behaviour caused by it (McEwen, 2000). As mentioned in the Introduction, the survey conducted by Klinck *et al.* showed that the negative behavioural side effects in dogs suffering from dermatological conditions were significantly related to corticosteroid therapies and not to the level of pruritus, as reported by owners, and this therefore supports our hypothesis (Klinck *et al.*, 2008).

In this preliminary study, the main goal was to find items for future investigation through a controlled survey and therefore owners were prompted to use their own words to describe their dogs' behaviours in an open and articulate way. Among the positive cases, most owners spontaneously reported several important behavioural changes after the first general questions (Table 2.1 Q1 and Q2). These questions could easily be included in routine follow-up monitoring in practice and may help alert practitioners to potential risks they need to be aware of, especially given that, in several cases, treatment was associated with aggression and an actual bite in two incidences.

Most of the dogs involved in the study suffered from dermatological problems and were

treated with oral corticosteroids, methylprednisolone and prednisolone, with similar dose ranges and therapeutic schedules. These started with a higher dose in the first week of therapy and continued with half the dose for another week and, finally, with the same half dose every two days for variable periods of time. It is worth noting that the reported behaviour changes seemed to appear quite early during corticosteroid treatments and this seems to be consistent with data reported in human studies (Warrington and Bostwick, 2006). Two owners (Dogs 20 and 29) explicitly reported a stronger association with corticosteroid treatment rather than the clinical condition for which the medication was prescribed, and this might be important since the latter could be a confounding factor in many case reports. Nonetheless it must be acknowledged at this time, that owners may easily perceive relationships when none actually exist, especially since some may perceive corticosteroids as “dangerous” drugs (Cullen *et al.*, 2006).

The co-administration of other therapies further complicates the interpretation of the results. It has been showed that some antibiotics can affect behaviour, for example penicillin and its analogues have been associated with sedation and anxiety (Pies, 1999; Turjanski, 2005), while quinolones, widely used in dogs and cats might cause, in rare cases, restlessness and irritability or, on the contrary, lethargy (Sternbach and State, 2009; Turjanski, 2005). Many other drugs that are often used in dogs, like antiparasitic products both for topical or oral use can have effects on dogs’ behaviour (De Souza Spinoso *et al.*, 2000; Flório *et al.*, 1989). For example it was shown that amitraz, often used topically in dogs, can have central depressant effects (Flório *et al.*, 1989).

Another example about an antiparasitic drug commonly used in dogs is ivermectin, an agonist for the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) that can be administered orally, topically or by injection and can have serious nervous side effects such as depression, coma and even death. Collie dogs are particularly sensitive to this drug and minimal doses can cause severe neurological side effects (Edwards, 2003), but other more subtle behavioural side effects were observed in mice that became significantly more active in an open field exploration test during ivermectin treatment than before treatment, and also showed a greater acoustic startle amplitude compared with non treated mice (Davis *et al.*, 1999). It is reasonable to suggest that exogenous corticosteroid dosage might influence the onset of behavioural side effects and, in our sample, dose ranges varied and questions were directed generally to ob-

tain information about changes in behaviour on and off therapy, without any specific investigation about dose related effects. Dose and type of corticosteroid employed should be more thoroughly considered in future studies, along with other elements related to the pharmacokinetics of these drugs; for example unbound serum prednisolone levels are higher during periods of hypoalbuminemia (Wolkowitz *et al.*, 1990). Investigating the effect of dose of corticosteroid is a particularly challenging issue because they are often prescribed in dogs with changing titrations and are adjusted according to desired effect. A more specific investigation at different dosages would be useful in order to be able discern any possible correlation, as has been reported in humans, between dose and behavioural disturbances, although even the human data are not consistent (Felder-Puig *et al.*, 2007; Hall *et al.*, 2003).

Other factors to consider include genetic influences on negative feedback mechanisms that are involved in stress response (Gómez *et al.*, 1998), and the rhythmicity of HPA activity. Cortisol is secreted in a pulsatory fashion; circadian patterns, similar to the ones that have been demonstrated in humans, are difficult to verify in dogs probably because of the very different housing and management conditions of individual dogs and groups of dogs (in shelters, laboratories, in breeder kennels in households and so on), but it seems reasonable to suggest that there are patterns of diurnal activity, depending on dog management conditions (Kolezvská *et al.*, 2003). Feeding appears to be one of the most important regulatory elements for the synchronization of HPA activity (Leal and Moreira, 1997). Exogenous corticosteroid might therefore interfere with the normal physiology of HPA axis depending on the management of dogs and we cannot exclude that some effects were influenced by external factors such as the time of feeding or the housing conditions. The effects might also be indirect; for example, the appetite stimulant effects of corticosteroids are widely recognised, and if a dog is hungrier, it may be more prone to guard food and associated aggression. Other indirect relationships might also exist with some of the other reported signs. The above issues suggest caution in the interpretation of the results of this first survey but the results encouraged further investigation given both the strong theoretical basis for corticosteroids increasing vigilance and biasing sensitivity towards aversion.

2.5 Conclusion

In this chapter, semi-structured interviews provided useful preliminary information about behavioural effects of corticosteroid drugs in dogs, and areas of future investigation were identified. The outcomes of the interviews reported here have been used in the next phase of this research, to investigate more rigorously the possible relationship between these signs and corticosteroid drug use in dogs.

Chapter 3

The Effects Of Corticosteroid Drugs On Dog Behaviour: Comparative Survey

In this chapter the information gathered in the preliminary survey was used to build a more structured questionnaire to survey a larger number of respondents. In order to have controls, dog owners with dogs receiving treatment with a range of corticosteroid preparations and dog owners with dogs receiving treatment with other drugs, were asked to complete the questionnaire. Dogs under corticosteroid treatment were reported to show changes in their behaviours that were consistent with the findings of the preliminary study reported in Chapter 2.

3.1 Introduction

In the first phase of our research we wanted to explore what dog owners might have noticed when their dogs were receiving treatment with corticosteroid drugs. From the literature, we already knew that in other species a number of behaviours related to avoidance, fear and negative emotion in general have been shown to appear or increase when animals and humans are treated with corticosteroids (Kajiyama *et al.*, 2010; Korte, 2001). Cognitive changes such as memory impairment have also been described in laboratory animals, suggesting that corticosteroids might cause changes related with both emotion and cognition (Henckens *et al.*, 2012; Kim and Haller, 2007). Because corticosteroids might mimic the effects of organisms' stress responses, it is not surprising that treatment side effects such as increased avoidance, fear and aggression seem to be more evident in already distressed individuals (Brown and Chandler, 2001; Panwar and Lassi, 2011; Wolkowitz *et al.*, 1990). One of the main challenges, when investigating the effects of drugs on behaviour, is to identify to what extent treatment contributes to the onset or worsening of signs of negative affective states in subjects that are also suffering physical discomfort, that increases distress *per se* (Blackburn-Munro and Blackburn-Munro, 2003). This survey aimed to investigate the effects of corticosteroid therapies on dog behaviour through the analysis of questionnaires completed by dog owners which included the use of other drugs used for similar conditions for comparative purposes. The preliminary survey established the primary areas likely to be of importance (see previous chapter), and informed the design of the present study questionnaire, which also allowed the introduction of a number of additional controls to reduce some of the risks associated with responder bias.

3.2 Materials and Methods

The introductory part of the questionnaire gathered demographic data relating to both the owner and their dog, information about the drugs being given to the dog at the time of survey (such as type of drug, time of administration and doses) and information about the type of condition/disease for which it was being used. The respondents were asked to mention all drugs taken in the same period for the same or other concomitant conditions. Twelve double items of the questionnaire were related to the animal's behaviour (Appendix A). This was completed by dog owners with dogs receiving or having recently received drug treatment, preferably for dermatological or orthopaedic conditions. This part of the questionnaire was informed by the results of the previous survey, described in Chapter 2. Seven of the 12 items were selected on the basis of the results of the previous survey (Chapter 2), with five further questions relating to other behavioural changes not identified in the previous survey selected among the behaviours that most frequently cause complaints by dog owners (Adams and Clark, 1989; Beaver, 1994). These were inserted partly as “fillers” and to aid validation of target effects. Items in questionnaires are listed to achieve information about a particular topic and inserting questions that “hide” the real intent of the questions is likely to improve the reliability of the results (Domino and Domino, 2006). The questionnaire was published via the Internet in both English and Italian, with a paper version also distributed to Italian veterinary clinic clients. Questionnaires were back translated by independent mother tongue translators to assess the consistency of the two versions. The items were scored on a seven point scale with two scores for each question posed: one score for the respondent's perception when the animal was receiving pharmacological treatment for the condition and one for when the animal was not receiving pharmacological treatment (Figure 3.1).

WHEN YOU ANSWER THE FOLLOWING QUESTIONS, YOU ARE KINDLY REQUESTED TO THINK, AT FIRST, ABOUT THE BEHAVIOUR OF YOUR DOG BEFORE STARTING THE DRUG AND PUT AN X WHERE APPROPRIATE IN THE UPPER UNSHADED STRIP. THEN YOU SHOULD THINK ABOUT YOUR DOG'S BEHAVIOUR WHILE YOUR DOG IS TAKING THE DRUG AND PUT AN X WHERE APPROPRIATE IN THE DARKER, LOWER STRIP

Q1. Play behaviour. Some dogs are very motivated to play with people, other dogs or toys. On a scale from 1 to 7 where 1 is 'not very playful' and 7 is 'very playful' how would you rate your dog's behaviour?

Not very playful ←————→ *Very playful*

Without drug							
With drug							

1 2 3 4 5 6 7

Figure 3.1: Example of question and scoring system used in owner questionnaire:
Q1 Play Behaviour.

Questions are illustrated in Table 3.1 and Items 1 (Play behaviour), 5 (Attention seeking), 7 (Obedience), 8 (Guarding behaviour) and 12 (Mounting behaviour) were added as additional fillers.

QUESTIONS	DETAILS OF QUESTIONS
Q1	Some dogs are very motivated to play with people, other dogs or toys. On a scale from 1 to 7 where 1 is “not very playful” and 7 is “very playful” how would you rate your dog’s behaviour?
Q2*	Thinking about your dog’s temperament, how would you define its nervousness/restlessness on a scale from 1 to 7 where 1 is “very nervous and restless” and 7 is “very calm”?

Table 3.1: Continued on next page

QUESTIONS	DETAILS OF QUESTIONS
Q3*	Thinking about your dog's general responses, for example, in the presence of unknown people or of new, unknown stimuli (sounds, loud voices, unknown contexts, unknown animals or children...), on a scale from 1 to 7 where 1 is "extremely fearful and insecure" and 7 is "very confident" , how would you rate your dog?
Q4	Thinking about your dog's behaviour when there is food around, on a scale from 1 to 7 where 1 is not at all aggressive and 7 is very aggressive in the presence of food, how would you rate your dog?
Q5	Some dogs tend to be very insistent and seek physical contact with owners by jumping up, snapping, scratching with a front paw, whining or barking: on a scale from 1 to 7 where 1 is "no attention seeking behaviours" and 7 is "frequent and intense attention seeking behaviours", how would you rate your dog?
Q6	Some dogs bark at any time, night and day, some others bark only in exceptional occasions. On a scale from 1 to 7 where 1 is "rare barking" and 7 is "frequent and intense barking", how would you rate your dog's behaviour?
Q7	Some dogs are very obedient, for example they come when called and go to bed when asked, while some others are less easily controlled. On a scale from 1 to 7 where 1 is "not at all obedient" and 7 is "very obedient", how would you rate your dog's behaviour?

Table 3.1: Continued on next page

QUESTIONS	DETAILS OF QUESTIONS
Q8	Some dogs are very predisposed to guarding behaviour and tend to threaten people by barking and growling, some others are friendly with everyone and don't show any guarding behaviour. On a scale from 1 to 7 where 1 is "no guarding behaviour" and 7 is "intense & frequent guarding behaviour", how would you define your dog's behaviour?
Q9	Some dogs tend to startle very easy, for example when they hear a sound or are suddenly touched. In these cases they can react by fleeing, getting jumpy or showing aggression. On a scale from 1 to 7 where 1 is "low/rare startle response" and 7 is an "excessive and very frequent startle response", how would you define your dog's behaviour?
Q10	Some dogs tend to react aggressively if someone tries to touch them or come close while they are resting. These dogs can become aggressive whenever the owner tries to brush them, medicate them or even simply tries to pet them. On a scale from 1 to 7 where 1 is "never aggressive when disturbed/restrained" and 7 is "very aggressive when disturbed/restrained", how would you define your dog's behaviour?

Table 3.1: Continued on next page

QUESTIONS	DETAILS OF QUESTIONS
Q11	Some dogs have a marked tendency to avoid people or situations that are unknown or unfamiliar, for example they tend to leave the room when unknown guests arrive or when people scream or there are loud noises. On a scale from 1 to 7 where 1 is “no tendency to hide or avoid people or situations” and 7 is “High tendency to hide or avoid people or situations”, how would you rate your dog’s behaviour?
Q12	Some dogs can show a tendency to mount people (children and adults) or other dogs, often of the same sex. On a scale from 1 to 7 where 1 is “no tendency to mount” and 7 is “high tendency to mount” how would you rate your dog’s behaviour?

* reversed scale

Table 3.1: Owner questionnaire. Verbatim of questions. Scale 1-7 illustrate the level of expression of the investigated behaviour.

3.2.1 Data analysis

Summary descriptive statistics was calculate initially. A Spearman’s correlation test was used in a first analysis to identify possible correlations between changes in the investigated behaviours and use of corticosteroid drugs. For the purpose of this first analysis the sample was divided into two main groups, one group was composed of dogs that used corticosteroids, alone or with other drugs, and the other group was composed of dogs that assumed other drugs.

Responses were then collated and analysed using a repeated measures multivariate GLM . In this analysis, treatment related effects on behaviour when on and off drug were considered as dependent measures; type of treatment (divided into 3 categories: corticosteroids, corticosteroids and other drugs, only drugs other than corticosteroids), duration of treatment (divided into 5 categories: 1 week, 1-2 weeks, 2-3 weeks, 2-4 weeks, more than 4 weeks), the reason for treatment (divided into 3 categories: dermatological conditions, orthopedic conditions and others) were considered independent

factors. This first multivariate analysis was made to test drug effects within-subjects, and since only treatment type was found to be a significant factor and there was great variation in the baseline value of subjects, a univariate GLM was then used to examine the difference between behaviour when on and off treatment versus treatment type, with post hoc pairwise comparisons corrected for multiple testing by means of Bonferroni correction. Data were analyzed using SPSS 21.

3.3 Results

By the close of the survey in February 2011, 98 questionnaires had been completed correctly by dog owners and were considered suitable for analysis. Dogs were from a variety of breeds and genders, and aged between 1-14 years. Figure 3.2-3.4 shows breed, age, gender and reproductive state distributions of dogs (see Appendix D for description of breed groups).

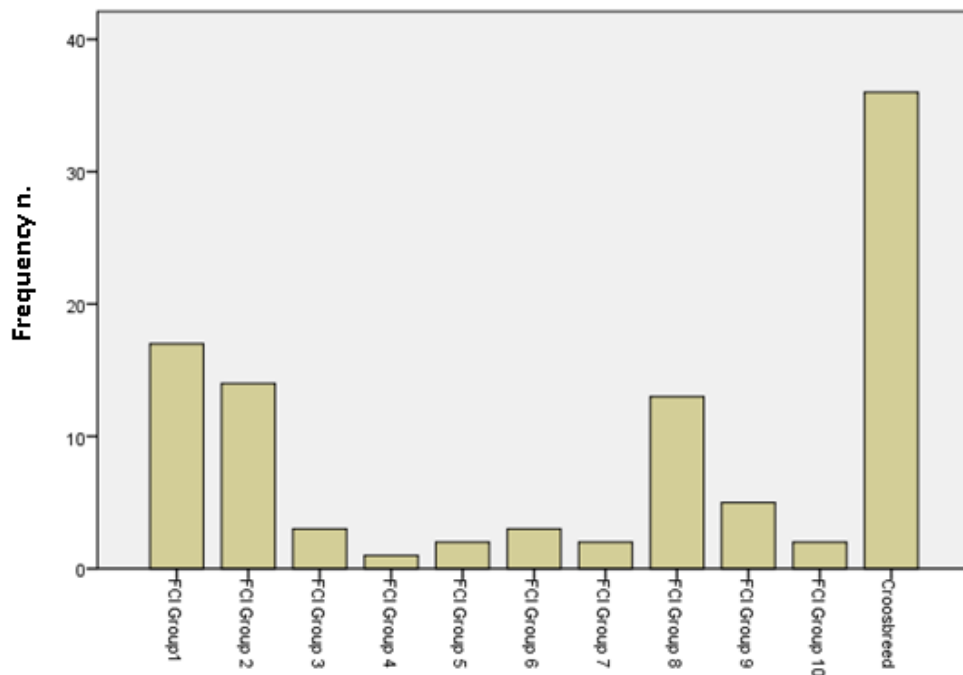


Figure 3.2: Breed distribution of dogs, according to the classification of the Fédération Cynologique Internationale (FCI): details in Appendix F).

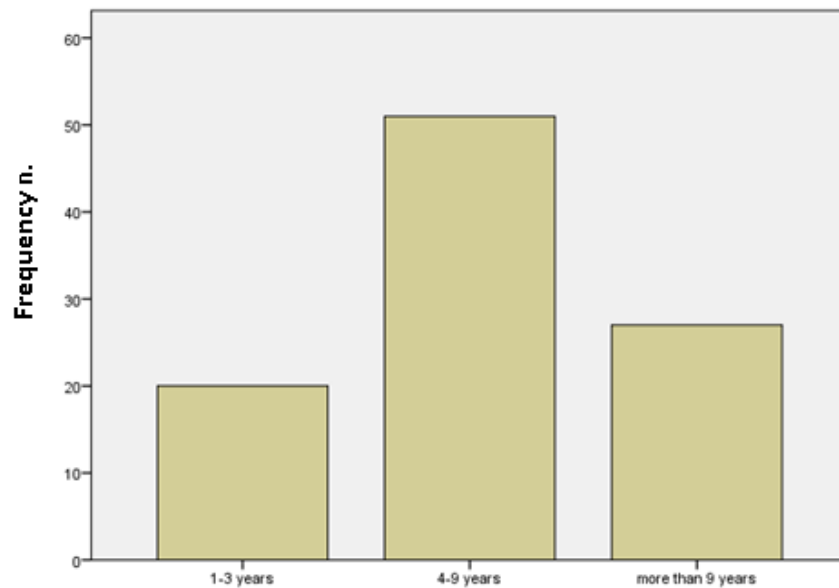
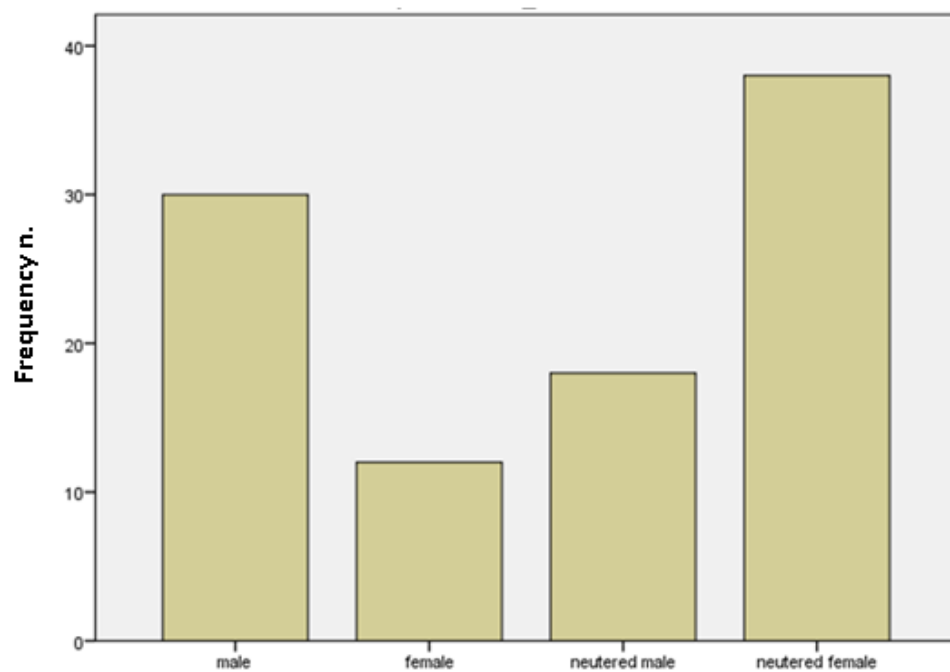


Figure 3.3: Age distribution of dogs divided into three main categories.



Male = non neutered males; Female = non neutered females.

Figure 3.4: Gender distribution of dogs with the indication of their reproductive state.

Reasons for treatment were dermatological conditions ($n = 55$), orthopedic condi-

tions ($n = 36$) and other kinds of condition that included respiratory, gastrointestinal and immune diseases that were grouped together because of the very small number of subjects in each category ($n = 7$). Treatment duration varied from one week of treatment to long term maintenance treatment. Of the sample of 98 dogs, 44 received corticosteroids and 54 received only other medications, mainly antibiotics ($n = 20$) and non-steroidal anti-inflammatory drugs ($n = 28$), with a small proportion on other drugs ($n = 6$) that included immunosuppressive drugs, antacid and antiemetic drugs. Of the 44 dogs that received corticosteroids, 23 also received other drugs, mainly antibiotics. The 44 dogs receiving treatment with corticosteroids were subject to a variety of corticosteroid preparations, but mainly prednisone/prednisolone ($n = 32$) and methylprednisolone ($n = 7$). Two dogs received betamethasone and three dogs received dexamethasone. Corticosteroid drug doses were between 0.1-1.2 mg/kg for prednisone and prednisolone, between 0.5-1.5 mg/kg for methylprednisolone, 0.05 mg/kg for betamethasone and 0.1 mg/kg for dexamethasone.

A Pearson correlation test revealed a significant correlation between corticosteroid treatment and differences in eight of the investigated behaviours, as reported by owners, (see Table 3.2).

Response Items	Pearson Correlation	Significance (2-tailed)
Play**	- 0.325	0.001
Nervousness**	- 0.292	0.004
Fear**	- 0.433	0.000
Food Aggression**	0.316	0.002
Attention Seeking	0.013	0.898
Barking**	0.302	0.003
Obedience	- 0.105	0.306
Guarding	0.001	0.993
Startle Reactions**	0.297	0.003
Irritable Aggression**	0.283	0.005
Avoidance*	0.236	0.019
Mounting	0.081	0.429

Significant items are in bold. ** = $p < 0.01$; * = $p < 0.05$ *

Table 3.2: Correlations between the differences in behaviours on and off treatment and corticosteroid treatment as reported by owners (n. 98 dogs).

Data were not normally distributed, but still suitable for analysis of variance (Ballinger, 2004; Taylor, 2011).

The repeated measures multivariate GLM analysis showed that the only significant factor related to a change in the behaviour of dogs was the treatment used. The treatment administered had a statistically significant effect on the response to eight items. Five behaviours, Play ($F = 6.525$), Nervousness ($F = 6.130$), Fear ($F = 13.112$), Startle Reactions ($F = 5.705$), Irritable Aggression ($F = 5.080$) – significantly changed with $p < 0.01$; three behaviours, Food Aggression ($F = 4.793$), Barking ($F = 4.330$), Avoidance ($F = 4.463$) – significantly changed with $p < 0.05$.

Post-hoc pairwise comparisons showed that treatment with corticosteroids (44 dogs) produced significant changes in behaviour for the items Play ($p < 0.01$), Nervousness ($p < 0.01$), Fear ($p < 0.01$), Food Aggression ($p < 0.05$), Barking ($p < 0.05$), Startle Reactions ($p < 0.01$), Irritable Aggression ($p < 0.01$) and Avoidance ($p < 0.05$). By contrast, treatments without corticosteroids (54 dogs), produced no significant changes ($p > 0.05$) in response to any item but playfulness ($p < 0.01$), which showed a change in the opposite direction with dogs becoming more playful when treated with other drugs (Table 3.3).

RESPONSE ITEM		CG GROUP		OG GROUP	
		Off	On	Off	On
Play behaviour	Mean	4.80	4.05**	3.80	4.63**
	(± SD)	(1.82)	(1.71)	(1.90)	(1.85)
Nervousness	Mean	4.57	3.75**	4.69	5.02
	(± SD)	(1.56)	(1.92)	(1.78)	(1.55)
Fear	Mean	4.89	3.95**	4.54	4.94
	(± SD)	(1.48)	(1.72)	(1.61)	(1.37)
Food Aggression	Mean	2.00	2.57*	2.20	2.07
	(± SD)	(1.45)	(2.11)	(1.56)	(1.37)
Attention Seeking	Mean	3.91	4.14	3.93	4.15
	(± SD)	(1.70)	(1.84)	(1.86)	(1.77)
Barking	Mean	2.73	3.43*	2.74	2.67
	(± SD)	(1.69)	(2.05)	(1.75)	(1.78)
Obedience	Mean	5.18	4.91	5.07	5.04
	(± SD)	(1.48)	(1.60)	(1.33)	(1.29)
Guarding	Mean	3.39	3.50	3.20	3.31
	(± SD)	(1.87)	(2.05)	(2.10)	(1.92)
Startle reactions	Mean	2.84	3.77**	3.19	3.06
	(± SD)	(1.68)	(2.07)	(1.83)	(1.62)
Irritable Aggression	Mean	1.93	2.43**	2.07	1.96
	(± SD)	(1.47)	(1.83)	(1.46)	(1.30)
Avoidance	Mean	2.27	2.73*	2.29	2.11
	(± SD)	(1.80)	(2.05)	(1.48)	(1.42)
Mounting	Mean	1.80	1.75	2.02	1.80
	(± SD)	(1.29)	(1.48)	(1.56)	(1.19)

* = $p < 0.05$; ** = $p < 0.01$ (Significant differences within groups)

CG = Corticosteroid Group; OG = Other Group

Table 3.3: Reported changes in behaviour score on and off different treatments. Scale 1-7 represent the expression of the behaviour. Questions about nervousness and fear had reversed scales.

3.4 Discussion

The results reported here are highly consistent with those reported in the previous chapter, using a different methodology. All the behaviours that were reported to change under the influence of corticosteroid drugs in the preliminary survey were found to change significantly in the present study, adding weight to the reliability of these reported effects. In addition, one further item, not previously reported, but also possibly influenced by negative affect i.e. a change in play behaviour, was also found to be reportedly reduced under corticosteroid treatment. The robustness of these findings is further enhanced by the finding that the other four items (attention seeking, obedience, guarding and mounting) introduced as fillers did not show significant changes associated with the use of corticosteroid therapies.

The additional discovery of an effect on play is important as play is considered to be a useful indicator of animal welfare, with animals not playing if they are in a distressed state (Boissy *et al.*, 2007; Held and Špinka, 2011; Oliveira *et al.*, 2010). The finding is all the more interesting as corticosteroids are widely used to relieve pain and irritation in veterinary practice, and it might be predicted that their use would increase playfulness as a result. However, these results suggest that their effects on affective state can outweigh these beneficial effects and that their value in this context might be largely related to increasing arousal rather than a positive affective state, as might be often assumed. The question about play behaviour was inserted as a filler but it worth notice that in the preliminary survey (see Chapter 2) two owners reported a decrease in their dogs' activity levels and this might have included a decrease in play behaviour although it was not specifically mentioned.

The effect of corticosteroids on increasing nervousness was identified in the survey illustrated in Chapter 2, with six owners (out of a sample of 31) describing that their dogs tended to be more agitated and restless. In the current study, dogs receiving corticosteroids were also reported to be significantly more nervous and restless, more fearful/less confident, and more prone to startle when on treatment and such a response was not revealed in relation to other medications. Agitation and restlessness have been described frequently in humans treated with corticosteroids (Benyamin *et al.*, 2008; Warrington and Bostwick, 2006). Endogenous corticosteroids are important factors in a range of stress responses and are involved in the onset of avoidance behaviours, probably by inducing chemical changes that predispose the subject to the onset of reactions related to fear and anxiety (Korte, 2001; Rodrigues *et al.*, 2009; Steimer,

2002). We hypothesize that, from a biological perspective, this central response is possibly a more reliable consequence than the behavioural output, and may offer a better explanation of the impact of corticosteroids on affective state. Although a rise in endogenous corticosteroids is often attributed to the presence of aversive stimuli, it is well recognized that a rise also occurs at other times which might be associated with positive affect, e.g. during sexual arousal (De Kloet *et al.*, 1999; McEwen, 2007). This has led some to speculate that corticosteroid levels are simply a reflection of arousal. However, an interpretation based on increased cognitive sensitivity to aversion may be more useful, since even at times of intense positive stimulation, this bias is adaptive, because these are also occasions when the animal may be more vulnerable to predators due to its focused attention on positive salient stimuli. Therefore, the release of corticosteroids at this time not only facilitates the necessary increased arousal, but also serves to reduce the risk of harm, by simultaneously predisposing the animal to threat vigilance. In this context it is worth noting that the startle response (which was reportedly increased in this study) has been used as a specific response modulated by negative affective states both in laboratory animals and human beings (Angrilli *et al.*, 1996; Dreissen *et al.*, 2012; Gresack and Risbrough, 2011; Lang *et al.*, 1990). Further empirical investigation of this hypothesis is possible since it has been found that animals in negative affective states are also more likely to respond negatively to ambiguous stimuli (Burman *et al.*, 2011; McNaughton and Corr, 2004; Mendl *et al.*, 2010; Paul *et al.*, 2005).

The tendency to behave aggressively in the presence of food is consistent with this hypothesis but also with other known effects of corticosteroids. Many dogs receiving exogenous corticosteroids may have an increased appetite, eat and drink more (Sousa, 2009; McDonald and Langston, 1995). This could result in an increased perceived value of food, and tendency to defend it from possible competitors as a result. The concomitant generation of a negative affective state will also increase the risk of an aggressive response in the presence of ambiguous and potentially threatening stimuli.

Dogs under corticosteroid treatment were also reported to be significantly more prone to react aggressively when disturbed. In the preliminary survey described in Chapter 2, four owners described their dogs, during treatment with corticosteroids, showing the tendency to react aggressively when petted or just approached.

The way dogs respond to a social approach depends on several different elements, and

aggression at this time may be viewed as a response style associated with a wider range of stress-related responses. Studies in both humans and laboratory animals have shown that corticosteroids play an important role in the onset of aggressive responses (De Kloet *et al.*, 1996; Kim and Haller, 2007).

Subjects under corticosteroid treatment were also perceived to be significantly more prone to avoiding people or situations. The influence of corticosteroids on avoidance behaviour and learning has been reported in both laboratory animal studies and human case reports (Cottrell and Nakajima, 1977; De Kloet *et al.*, 1999; Medina *et al.*, 2007). This result is again consistent with the tendency for dogs under treatment with corticosteroids to be in a general negative affective state.

Owners also reported that dogs under corticosteroid treatment barked significantly more than when they were not receiving therapy, although no specific context or situation was defined for this behaviour. Vocalizations are part of dog communicative behaviour but can also be indicators of emotional arousal, either positive or negative and the further interpretation of this behaviour would need a description of the vocalization features, of the visual signals given by the dogs along with an evaluation of the context of the displayed behaviour. Nevertheless, it was reported that an increased tendency to bark can be observed in dogs that are fearful or distressed and intense, frequent barking is perceived as a problem by dog owners (Beerda *et al.*, 2000; Landsberg and Akermann, 2003), and so owners should be warned of this potential side effect too.

No effect of corticosteroid administration on the fillers relating to obedience, attention seeking, guarding or sexual behaviour was found. Filler items are often used in the design of questionnaires in psychology to prevent bias (Domino and Domino, 2006). The fillers used here were chosen following consideration of the behaviours that most frequently cause complaints by dog owners and which are part of the behavioural repertoire of most pet dogs (Adams and Clark, 1989; Beaver, 1994; Lindell, 2009; Wells and Hepper, 2000). These behaviours were not mentioned by dog owners in the previous survey and so the lack of perceived significant changes in the present survey supports the specificity of the reporting by owners in the current study. Overall, these results provide further evidence that in dogs, as in other species, corticosteroid drugs can bias cognition and change behaviour, but further empirical studies are needed in order to confirm these findings. Nonetheless in the absence of evidence to the contrary, the “precautionary principle” should be followed, with advice given on these

potential side effects for best practice.

Except in the management of acute episodes, corticosteroid drug treatments in dogs are typically given for one or more weeks depending on the type and severity of disease, and the dose is gradually reduced; some treatments require a prolonged or even lifelong treatment (Sousa, 2009; O'Neill *et al.*, 2012). The decision to consider the length of the drug therapy was partly related to the need to have reliable data and to exclude cases of single administration, for example for dealing with acute allergic reactions. However, in the clinical reports of the psychiatric side effects of glucocorticoid drugs in humans, it seems that these can be both dose-dependent and time dependent (Bender *et al.*, 1991; Brown *et al.*, 1999). The issues of dose-related and time-related effects were not the specific focus of this investigation, and although neither factor was found to be significant in the initial statistical analysis, their potential relevance should not be dismissed on the basis of this study. Further investigation using multiple observations at different times on individual dogs are necessary, in order to build chronological data for analysis. The possible influence of different types of corticosteroid drugs could not be investigated because about 90% of the corticosteroid drugs were very similar molecules (prednisone, prednisolone and methylprednisolone), but this aspect might be worth investigation in future studies.

All dogs involved in the survey were in treatment for different conditions and the effects of disease on behaviour have been widely reported and studied (Larson and Dunn, 2001), for example, the behavioural effects of cytokines involved in the immune response, such as decreased exploratory behaviour and increased avoidance (Anisman and Merali, 1999). Problematic behaviour was considered in the preliminary survey in Chapter 2, where case studies were reported, with two dog owners reporting an association with corticosteroid treatment rather than the clinical condition for which the medication was prescribed. The treatments in these two dogs were discontinued because of the onset of behavioural problems after a few days of corticosteroid therapy and both dogs, although still in bad physical condition, reportedly stopped showing the unwanted behaviours as a result. It is worth noting that in the present survey different groups were defined on the basis of the treatment used but only the corticosteroid group showed significant behavioural changes.

Owner reports of individual dog behaviours might be biased by pre-existing personal opinion, expectation, cultural attitudes and beliefs. Corticosteroids are often portrayed in popular media as dangerous drugs and owners' evaluations might therefore

be influenced by such a perception rather than by direct observation of their dogs' behaviour (Cullen *et al.*, 2006). This sort of bias is probably the main risk of surveys based on the opinions of dog owners, but there is also an important potential advantage associated with gathering information about dogs' behaviour from owners associated with their experience in a range of contexts (Wemelsfelder, 1997; Wiseman *et al.*, 2001). The direct testing of dogs may be more reliable in terms of collection of data but has the limitation of context specificity. However, given the results established so far, the next logical step is to see if the perceptions reported here are replicated in a more objective and controlled behavioural test setting.

3.5 Conclusion

The results presented in this chapter, are consistent with previous findings and contribute additional evidence to suggest that exogenous corticosteroid treatment can influence the onset of avoidance responses and aggressive responses in dogs. This can be explained by a tendency for these drugs to bias sensitivity towards aversion in dogs that is also associated with an increase in vigilance.

Chapter 4

Effects Of Exogenous Corticosteroids On Dog Behaviour: Behavioural Tests

In this chapter, a phase of this research that involved behavioural testing of dogs on corticosteroid therapies is described. This phase is intended to provide “dog-based” objective data about the effects of corticosteroid drugs on dog behaviour through the direct testing of dogs in treatment with these drugs. A control group of dogs was also tested using the same methodology. Dogs in treatment with corticosteroid drugs were less explorative and eat less pieces of food compared with the control group.

4.1 Introduction

After having investigated the possible behavioural side effects of corticosteroid drugs in dogs indirectly through owners’ opinions, the following step was to carry out a behavioural test in order to have more objective data recorded directly from the dogs themselves that could support our hypothesis that corticosteroid drugs in dogs can bias sensitivity towards aversion. Directly testing dogs gives the possibility to measure their behaviour in a more objective way, compared with the subjective evaluations of dogs’ owners although , with the survey approach, the owners’ responses might provide information about complex behaviours in different contexts (Svartberg, 2005), and main limit of directly testing dogs is the narrow range of investigated contexts. Testing dogs also implies ethical issues, for example dogs might be scared by social and environmental stimuli used to elicit their behavioural responses and test experience might cause the onset of negative associations that might last and possibly elicit future avoidance responses. These issues had to be addressed first in the phase of test design and then during the development of test attempts in order to select appropriate setting and stimuli (see Appendix C).

From the results of the previous surveys, reported in Chapters 2 and 3, we knew that dogs on corticosteroid drugs might become less playful, more nervous, more fearful, more aggressive in the presence of food, bark more, have an increased tendency to startle, be more irritable and increase their tendency to avoid unusual situations. Our hypothesis was that these changes in behaviour were related with increased negative affective states and arousal. Setting an experimental condition that would enable us to detect the presence or increase of a negative affective state in dogs, but without endangering their welfare, was the major challenge.

Behavioural tests intended to demonstrate changes in behaviour that reflect differ-

ences in affective state as a consequence of exposure to pharmacological treatments have been largely developed and used in laboratory animals (Ardayfio and Kim, 2006; Handley and Mithani, 1984; Kajiyama *et al.*, 2010). For example the reactions of male rats to spatial novelties in relation with different levels of corticosterone were investigated in an open field test, which is an arena with walls to prevent escape. This interesting experiment investigated the U-shaped effects of corticosteroids, where very low levels and very high levels of these hormones seem to have similar effects on reactivity. They found that adrenalectomized rats increased their reactivity to novel objects in the arena but also high doses of corticosterone produced similar patterns of behaviour (Oitzl *et al.*, 1994). While the majority of studies about drugs that can affect behaviour in dogs have been conducted in owned dogs and are based on owners' opinions (King *et al.*, 2000; Landsberg *et al.*, 2008), more recently, behavioural tests have been used with laboratory dogs to demonstrate the effects of nutraceuticals on dog behaviour (Araujo *et al.*, 2010; DePorter *et al.*, 2012).

For instance, testing an increase or decrease of specific behaviours thought to be associated with the experience of negative emotions when exposed to aversive stimuli (e.g. fear of thunderstorms) was investigated by DePorter *et al.* with the purpose of testing the efficacy of a nutraceutical compound on thunderstorm phobia in dogs (De Porter *et al.*, 2012). This latter study was a blinded and placebo study and dogs were tested in an open field test. Activity levels were the primary outcome measure of negative emotions, with reduced inactivity during the administration of artificially reproduced thunder sounds taken to indicate a reduction in fear and anxiety. Inactivity duration, inactivity frequency, distance travelled and time spend close to the exit of the arena during the administration of the aversive sound were considered as related to freezing responses and attempt to escape and used as parameters to measure anxiety. They found that dogs in treatment were less inactive during the thunderstorm phase but no other significant differences were detected. A different study on laboratory dogs investigated the effects of Anxitane[®], another nutraceutical compound based on the amino acid L-Theanine (N-ethyl-L-glutamine), an analogue of L-Glutamine and L-Glutamate (Nathan *et al.*, 2009), on the apparent fear of unfamiliar humans (Araujo *et al.*, 2010). In this study, dogs that were characterized as being anxious were observed to show less interaction with an unknown human compared to the interaction level of control dogs and this parameter was used to test the effect of the nutraceutical. They found that dogs fearful of unfamiliar humans, when in treatment,

increased the frequency and duration of interactions as well as the time spent near the human in the test environment (Araujo *et al.*, 2010). The interpretation of affective state using levels of activity as parameters might have limitations and in the study of DePorter *et al.* (2012) dogs neither significantly increased their distance travelled nor significantly decreased the time spent close to the exit of the arena, suggesting that increased activity might have been related with increased sympathetic activity, a stress response (Katz *et al.*, 1981). In the same way, in the study of Araujo *et al.*, (Araujo *et al.*, 2010), behaviours generated by an approach-avoidance conflict might have influenced the amount of time spent near the human (Roth and Cohen, 1986). Both in humans and laboratory animals, behavioural inhibition can be associated with elevated corticosteroid levels (Cavigelli *et al.*, 2007) while behavioural responses to novel, surprising stimuli and resilience have been related with the presence of positive emotional states (Cavigelli *et al.*, 2007; Degnan and Fox, 2007; Turner *et al.*, 1996). As mentioned above, behavioural inhibition or increased activities should be cautiously evaluated because high levels of arousal and increased activity intended as increased tendency to explore in a positive way might be difficult to distinguish. The behavioural test presented here was designed to challenge dogs with a surprising stimulus that was also intended to be slightly aversive in the context of the test setting in order to directly observe unambiguous behavioural changes in dogs under treatment compared to a control group of dogs. The findings of the previous stages of this research provided the rational to design the behavioural test described in this chapter. The aim was to see if dogs on corticosteroid therapies were more avoidant and less explorative, as shown by the increase of negative motivated behaviour illustrated in the previous surveys. With this test we wanted to directly investigate possible changes in the behaviour of dogs in treatment with corticosteroid drugs and all the treated dogs were patients of veterinarians that were not directly involved in this research.

4.2 Materials and Methods

4.2.1 Subjects

Eleven dogs receiving (or due to receive) corticosteroid treatment and 11 control dogs were recruited and successfully completed two sessions of the behavioural test. Treatment dogs were recruited from the patients of veterinary practices in the North of Italy. Veterinarians were asked to propose dog owners that had received prescriptions of oral corticosteroid drugs for dermatological problems to participate in the research. Criteria for inclusion were that dogs had not been prescribed any other medication; the prescription dose range was within 0.4-0.5 mg/kg of prednisone or methylprednisolone every day. Control dogs were recruited from among the healthy patients of veterinary practices and clients of dog trainers.

Control dogs were tested twice in the same environment as the dogs on corticosteroids, with the same time interval between the two tests. Details of all subjects are given in Table 4.1.

Dog	Breed/type	GENDER	AGE	TREATMENT(*)
1	Collie	M (n)	9	0.5/kg prednisone
2	Dachshund	M	4	0.4/kg prednisone
3	Labrador	M	5	0.4/kg methylprednisolone
4	Golden retriever	F (n)	10	0.5/kg prednisone
5	Crossbreed	F (n)	4	0.4/kg prednisone
6	Cocker	M	9	0.4/kg methylprednisolone
7	Pitbull	M (n)	7	0.4/kg prednisone
8	Jack Russell	M	3	0.5/kg prednisone
9	Crossbreed	M	1	0.4/kg prednisone
10	Golden retriever	F	2	0.5/kg prednisone
11	Crossbreed	M	4	0.4/kg prednisone
12	Crossbreed	M (n)	9	No treatment

Table 4.1: Continued on next page

Dog	Breed/type	GENDER	AGE	TREATMENT(*)
13	Crossbreed	F (n)	2	No treatment
14	Crossbreed	M	2	No treatment
15	Pitbull	M (n)	2	No treatment
16	Shiba Inu	M	1	No treatment
17	German shepherd	F	1	No treatment
18	Crossbreed	F (n)	7	No treatment
19	Cocker Spaniel	M	6	No treatment
20	Border collie	M	5	No treatment
21	German shepherd	M	4	No treatment
22	Crossbreed	F (n)	1	No treatment

* = no dogs were receiving treatment in the first test trial, treatment refers to medication in use during the second test trial.

M = male dog; F = female dog, (n) = neutered.

Table 4.1: Dogs involved in the study.

The first behavioural test for treatment dogs occurred just before they started therapy, with a second taking place 6-7 days into the therapy, often just before the dose of corticosteroid started being reduced with a view to its withdrawal.

4.2.2 Test procedure

The tests were conducted in three different locations in order to accommodate the travel restrictions of clients, but the set-up was the same at each of these: a room of sufficient size to accommodate the experimental apparatus, with a chair for the owner at the opposite end of the room. The apparatus composed of a screen covered with a blanket that hid a loudspeaker system connected to a computer. Five pots were placed in front of the screen, 35 cm from the loudspeakers. The pots were placed in a way that enabled the researcher to put small pieces of food into them at the same time (Figure 4.1). For each dog, the kind of treat used during testing was indicated by the owner as being the dog's favourite. The same kind of treat was used in both

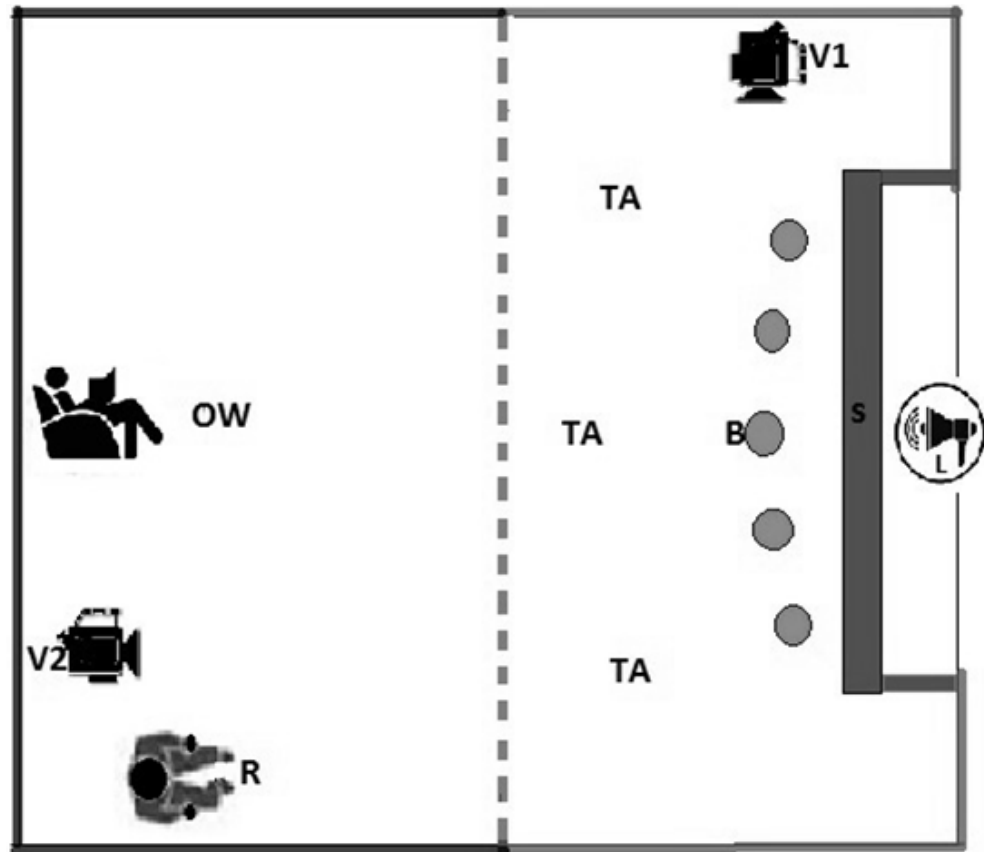
test trials for that subject. Two video cameras (Canon Legria HF R506) were used to record the dog's behaviour during testing for later behavioural coding. The video cameras were mounted on a tripod, one to one side of the room and the other at the back of the room. The part of the room within 150 cm of the screen was considered to be the "test area" and when dogs were within this area with evident interest in exploring it, the screen or the pots, their behaviour was considered as "exploring the test area". Exploring the test area included:

1. Sniffing: The dog overtly approached the floor, the bowls or the screen and appeared to inhale through its nose;
2. Exploring: Remaining in the test area watching towards the floor, the screen or the bowls;
3. Investigating the pots: approaching the pots with nose within 1 cm of pot and nose or muzzle inside pot.

Behaviours such as staying far from the screen, either close to the owner or at the opposite end of the room to the experimental apparatus, were considered as behaviours associated with not exploring the test area.

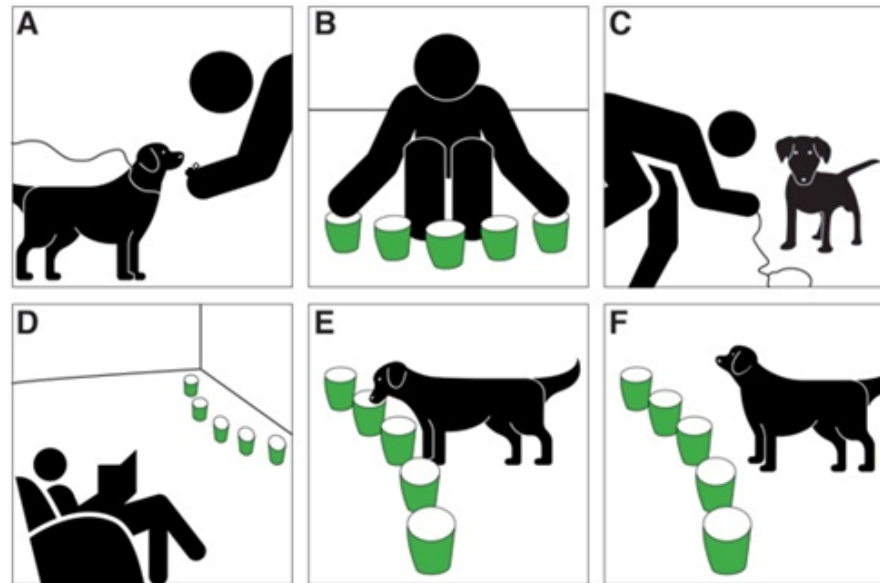
Dogs were brought into the test environment on a leash by their owners. In each test trial the owner was invited to calmly restrain the dog on the leash, sit and wear a pair of sunglasses to restrict eye contact between dog and their owner. The researcher showed the dog a few pieces of food and then put one small piece of food in each pot. After this, the researcher sat on a chair in a corner of the room, showing no overt interest in the procedure. The owner was instructed to unleash the dog and then behave in a neutral way, pretending to read a book provided by the researcher and completely ignoring the dog until a signal signifying the end of the test was given. The dog was left free to investigate the test area and take the treats from two pots. As soon as it started to approach the third pot, playback of a three second growl was started. Three types of dog growl recordings were used: small dog, medium dog and large dog growls, and these were allocated on the basis of the size of the dogs being tested (i.e. small test dog = small dog growl) in order to minimize the scaring effect of the growl. The growls were chosen because they had been recorded in the context of food guarding and used in a previous study (Faragó *et al.*, 2010). The dog's behaviour was then observed for two minutes. At the end of the test, the owner was asked to

call the dog and put it on its leash. This behavioural test procedure is illustrated in Figure 4.2.



V1 and V2 = Video cameras; TA = Test Area; S = Screen; B = pots disposition; L = Loudspeaker; OW = Owner position; R = Researcher position during the test.

Figure 4.1: Test setting.



A = the dog is shown the food; B = a piece of food is placed into each pot; C = the owner unleashes the dog; D = the owner is instructed to behave in a neutral way; E = the dog explores the pots; F = the dog reacts to the growl.

Figure 4.2: Behavioural test procedure.

4.2.3 Behavioural Observation

The video recordings of tests were analyzed using Solomon Coder (<http://solomoncoder.com/>). We considered the following behaviours for analysis:

- **Latency**

Time from release to the approach to the first pot (nose within 1cm of pot) (Latency 1);

Time from the growl/startle reaction to further investigation of pots (Latency 2).

- **Durations**

Time spent investigating the test area (TTA);

Time spent investigating the pots (TTP);

Time spent investigating the test area before the growl (Expl1);

Time spent investigating the test area after the growl (Expl2).

- **Startle reaction scores**

Grade 1. The dog responds with minimal, momentary re-orientation of head;

Grade 2. The dog responds with re-orientation of head, steps back;

Grade 3. The dog responds with re-orientation of head, steps back and takes a few seconds before coming back to the pot or leaves the test area and does not return within 2 minutes;

- **Food eaten**

The number of food pieces eaten by each dog in each test trial.

4.2.4 Data Analysis and Statistics

The first observer transcribed the video recordings of both test trials ($n = 22$) and scored them using the ethogram on two separate occasions to assess intra-observer reliability. The recordings from ten of the dogs (five treatment dogs and five control dogs) were randomly selected for their behaviour to be assessed by a second observer who was “blind” to the treatment allocation in order to evaluate inter-observer variability. Spearman’s correlation coefficient was used to measure pairwise correlation among raters.

Data from the video analysis regarding Latency 1, Latency 2, TTP, TTA, Expl1 and Expl2 were analyzed using SPSS 21. Data were not normally distributed and therefore an extension of the Generalized Linear Model (GZLM), Generalized Estimating Equations (GEE) was used in order to evaluate the results that accommodated correlated within-subjects data and allowed comparisons between subjects.

Startle reactions were evaluated for their severity according to the above descriptions. Eating of food was evaluated by counting the number of food pieces eaten by each dog during each test trial. Comparison between the control and treatment dogs for these two metrics was evaluated using Mann-Whitney U test at a given time point (e.g. either first or second test), with Wilcoxon’s Matched Pairs Signed-Ranks Test used to compare within groups between tests (first versus second test).

4.3 Results

The behavioural testing of dogs (11 sample dogs and 11 control dogs) ended in October 2013.

Spearman’s coefficient of correlation revealed statistically significant positive correlations between intra-observer ($n = 22$) and inter-observer ($n = 10$) measurements.

Intra-observer correlations were positive with $r = 0.994$ and $p < 0.01$. Inter-observer correlations were positive with $r = 0.996$ and $p < 0.01$ for all items.

GEE revealed no significant differences in Latency1, Latency 2, TTA, TTP, EXPL1 and EXPL2 between groups in the first test trial, before the “treatment” dogs had been placed on corticosteroids. In the second test trial, the total time spent investigating the test area (TTA) was significantly lower in the group of dogs treated with corticosteroids (unstandardized coefficient - $B = 25.309$; $\chi^2(1) = 6.157$; $p < 0.05$) compared with the control group of dogs.

In the second test trial, the exploration time after the growl of dogs (EXPL2) in the treatment group was significantly lower ($B = 26.18$; $\chi^2(1) = 6.600$; $p < 0.05$) compared with the same behaviour in the control group of dogs. Latency times (L1 and L2), time spent investigating the area before the growl (EXPL1) and the time spent investigating the pots (TTP) were not significantly different between the two groups ($p > 0.05$) [Latency 1: $B = -3.573$, $\chi^2(1) = 0.588$, $p = 0.443$; Latency 2: $B = -9.709$, $\chi^2(1) = 0.477$, $p = 0.490$; EXPL1: $B = -0.545$, $\chi^2(1) = 0.310$, $p = 0.577$; TTP: $B = 5.991$, $\chi^2(1) = 2.583$, $p = 0.108$].

Startle reactions in the first test trial were present in six dogs from the treatment group and nine dogs from the control group. Three dogs from the test group were graded at level 1 (S1) and four at level 2 (S2). Eight dogs from the control group were scored at level 1 (S1) and one at level 3 (S3).

In the second test trial seven dogs from the treatment group and nine dogs from the control group produced startle reactions (see Table 4.2). No significant differences between groups were found as far as startle reactions were concerned ($p > 0.05$).

In the first test trial, seven dogs from the treatment group ate all five food treats, two dogs did not eat any food and two dogs ate three pieces. In the first test trial, eight dogs from the control group ate all the food, two dogs did not eat any food and one dog ate three pieces.

In the second test trial, five dogs from the treatment group ate all the food, two dogs ate four pieces, two dogs ate three pieces and two dogs did not eat any food. In the second test trial, all dogs from the control group ate all the food. A Mann-Whitney U test revealed a significant difference between groups as far as number of pieces of food eaten was concerned ($z = -2.765$; $p = 0.028$) with control dogs eating more than treatment dogs.

Wilcoxon’s Matched Pairs Signed-Ranks Test revealed no significant differences within

groups in the two test trials ($p > 0.05$) for either startle or food consumption.

Dog	Trial 1 Startle score	Trial 2 Startle score	Trial 1 Food eaten	Trial 2 Food eaten
1*	—	—	5	5
2*	S1	S2	5	5
3*	—	—	5	5
4*	—	—	5	4
5*	S2	S3	3	3
6*	—	S2	5	0
7*	S2	S1	0	4
8*	S2	S2	0	0
9*	S1	S1	3	3
10*	S2	S2	5	5
11*	—	—	5	5
12	S1	S1	5	5
13	—	—	5	5
14	S1	S1	5	5
15	S1	S1	5	5
16	S1	S2	0	5
17	S1	S1	5	5
18	S3	S3	3	5
19	—	—	5	5
20	S1	S1	5	5

Table 4.2: Continued on next page

Dog	Trial 1 Startle score	Trial 2 Startle score	Trial 1 Food eaten	Trial 2 Food eaten
21	S1	S1	0	5
22	S1	S1	5	5

* = dogs receiving treatment with corticosteroids in the second test trial;
 Trial 1 Food = pieces of food eaten in trial 1; Trial 2 Food = pieces of food eaten in trial 2;
 S1 = The dog responds with minimal, momentary re-orientation of head; S2 = The dog
 responds with re-orientation of head, steps back; S3 = The dog responds with
 re-orientation of head, steps back and it takes a few seconds before coming back to the pot
 or never comes back.

Table 4.2: Startle reactions and pieces of food eaten by dogs in the two test trials.

4.4 Discussion

The results reported here from both studies are consistent with the preliminary findings described in Chapter 2 and the results of the survey reported in Chapter 3. Behavioural tests were included alongside the survey in order to provide, for the first time, objective and directly observed behavioural evidence of the effect of corticosteroid therapy on dog behaviour. Because our initial findings were interpreted to indicate that dogs on corticosteroid therapy were more avoidant, the test was designed to stimulate exploration of the test area with minimal challenges. We designed the test in a way that would have minimized the threat for the animal and asked the owner to be present in order to provide a safe point of reference for the dog. The owners were informed that they could interrupt the test in any moment if they felt that their dog was too distressed and this was a further guarantee of preservation of the animal's welfare. The introduction of a surprising, potentially negative stimulus in the form of the growl, had the purpose of testing both reactivity and avoidance tendency. Decreases in exploratory behaviour have also been associated with negative affective states (Cavigelli *et al.*, 2007; van Dijken, 1992; Rygula *et al.*, 2013), and this is likely to be the product of a negative cognitive bias associated with negative affect: when in a negative affective state the desire to seek new information is reduced and so the animal might avoid rather than explore open areas and novel stimuli (Burman *et al.*, 2011; Harding *et al.*, 2004). In the behavioural tests, types of corticosteroids

and drug doses were very similar within the treatment group. Cytokines involved in the immune response might explain behavioural effects (Anisman and Merali, 1999), such as decreased exploratory behaviour and increased avoidance reported by owners in the survey, but this would not explain the results obtained in the behavioural tests. Some dogs suffered from allergic dermatological conditions, that have been shown to induce the production of cytokines in different species including dogs (Gonzales *et al.*, 2013), when they were tested off treatment and no significant differences were found between treatment and control group dogs at this time; rather a significant decrease in exploratory behaviour between groups was observed only when treatment dogs were on corticosteroids.

Unlike the dogs receiving corticosteroid treatment, control dogs did not show a significant change in their exploratory behaviour, when receiving treatment. Comparisons between groups showed that the exploratory behaviour was lower when dogs were on corticosteroids. In laboratory animals, the administration of corticosteroids after a training session seemed to influence contextual fear memories and hippocampal long term potentiation (LTP), suggesting that they may enhance contextual fear memory consolidation via enhancing hippocampal LTP (Abrari *et al.*, 2009). The interaction between corticosteroids, memory and emotional arousal has been investigated in many studies (Rooszendaal *et al.*, 2006), showing that glucocorticoids interact with the noradrenergic system in brainstem noradrenergic cell groups that project to the basolateral amygdala. This may provide the neurological mechanism for the changes seen in the dogs on corticosteroids during their second visit to the experimental set up.

In the behavioural test, we found dogs on corticosteroids ate significantly less food compared with the control group, despite increased appetite being a well recognised side-effect of corticosteroid therapy (Sousa, 2009).

In the behavioural tests no significant differences in startle reactions were found in dogs treated with corticosteroids ($p > 0.05$). However, in the questionnaire study, dog owners reported an increase in their dogs' startling tendency when on corticosteroids and the startle response has been used as a specific response to assess a changed negative affective state in both laboratory animals and human beings (Angrilli *et al.*, 1996). This apparent discrepancy can be explained because startle responses have different motor features according to whether they are triggered by emotional or voluntary responses (Baschnagel *et al.*, 2007; Cook *et al.*, 1992; Davis *et al.*, 1982; Dreissen *et*

al., 2012; Koch, 1999; Lang *et al.*, 1990), and it may be that the two contexts focus on different types of startle. The setting of the behavioural test involved two types of salient stimuli, the food and the sound, in a framework that was supposed to be neutral. However, the size of startle reflexes might have been influenced not only by the underlying affective state of dogs but also by other ongoing attentional processes associated with the presence of food, which might differ between the two groups. The corticosteroids-related increase in appetite as previously discussed in chapter one and three (Sousa, 2009; McDonald and Langston, 1995), might have attenuated the startle response in the context of response to a growl while exploring food, since the animal was focused on eating rather than on potential threats. By contrast the results about startle reactions in the survey may reflect increases at other times, such as when the animal is already anxious about a potential threat.

As discussed in chapter one and three, subjective evaluation of dog behaviour might be biased but there is also an important potential advantage associated with gathering information about dog behaviour from owners associated with their experience in a range of contexts, since they know their own animals best (Wemelsfelder, 1997; Wiseman *et al.*, 2001). It is therefore important to support the findings from such surveys by showing convergent validity with more objective measures, as reported here. We decided to build a behavioural test in order to have more objective evidence of behavioural side effects of exogenous corticosteroids and, although the results of behavioural tests might appear to have a small magnitude compared with what is described in the literature for human patients, the decrease in exploratory behaviour has been interpreted as a sign of negative internal states of dogs in treatment. The study was designed in such a way that it did not endanger the dogs' welfare and therefore the presented stimuli were intended neither to scare nor to threaten the animals. In a real situation, a negatively motivated animal, in the presence of stressors of high magnitude (in general and compared with the ones proposed in the behavioural test), might show a much more evident response such as aggression or overtly fearful reaction. Direct testing of dogs in the behavioural test may be more reliable than indirect owner-based behaviour assessments, but is more labour intensive, especially when relying on clinical cases. This convergence, together with a consistency at the theoretical level, indicate that these results are robust and the effects reliable.

4.5 Conclusion

In this chapter, the direct testing of dogs showed that dogs on corticosteroids were less explorative compared with control dogs. Overall, these results indicated that in pet dogs, corticosteroid treatment at therapeutic doses can bias cognition and change behaviour. Therapeutic intervention with these drugs appears to increase sensitivity towards aversion and these findings are consistent with the theoretical level and the finding of the previous surveys presented in chapter two and three, indicating that these results are robust and the effects reliable.

Chapter 5

Corticosteroid Drugs And Dog Behaviour:

What Clinical Experience Can Tell Us

In this chapter the behaviour of 345 dogs, collected within the behavioural clinic caseload of the author between February 2012 and November 2014, were analysed. The goal of this retrospective survey was to investigate possible relationships between the affective states associated with the behavioural complaint of dogs and their previous history of treatment with corticosteroids drugs. Results showed that a history of corticosteroid treatment was a significant predictor of a range of behaviour problems associated with negative affective state.

5.1 Introduction

Throughout previous parts of this thesis, it has been highlighted how corticosteroid therapies might influence the behaviour of both humans and non-human animals. The results so far are consistent with the psychiatric side effects of corticosteroids reported in the human literature, but have focused on “mentally healthy” individuals. Although the results of the behavioural tests in the previous chapter might appear to indicate a small effect compared to what is described in the literature for human patients, the decrease in exploratory behaviour is potentially a sign of a negative internal state in dogs receiving treatment. It should also be noted that the study was designed in such a way so that it did not endanger the dogs’ welfare and therefore the stimuli used were intended neither to scare nor to threaten the animals, but to be ambiguously negative. In a real-world situation a negatively biased animal, in the presence of other stressors of higher magnitude or load might show a much more problematic response such as aggression or an overtly fearful reaction. Given these findings, it is worth asking the questions: “What proportion of dogs presented for different behaviour problems have had a history of treatment with corticosteroids?” and “Do corticosteroids appear to increase the risk of any complaints associated with sensitivity to aversion, e.g. fears and anxieties?”.

From the human psychiatric literature, we know that the negative psychological side effects of corticosteroid therapies can be more evident in patients that already have some psychiatric disturbance (Fardet *et al.*, 2012; Kenna *et al.*, 2011; Sirois, 2003; Ularntinon *et al.*, 2010). But it is not known what proportion of dogs with behaviour problems have taken corticosteroid drugs in the past. Such information will provide a more complete picture of the importance of the possible behavioural side effects of

these drugs, in particular in dogs predisposed to behaviour disturbance.

A study in humans conducted in the UK in 2012, described in Chapter 1 (Fardet *et al.*, 2012), showed how psychiatric disturbances in human patients were significantly related to corticosteroid treatments. Data were extrapolated from The Health Improvement Network (THIN) database between 1990 and 2008. THIN contains data about adult human patients registered with general practices. Patients with histories of corticosteroid treatments were compared with patients who did not receive such prescriptions. Human patients older than 18 years who had received at least one corticosteroid prescription were identified as exposed to corticosteroids (n. 372,696) and they were compared with two groups of patients that did not receive corticosteroids. The first group of unexposed patients was a randomly selected sample of patients that did not receive corticosteroid prescriptions (n. 1,224,984), the second group of unexposed patients did not receive corticosteroid prescription too, but had been diagnosed with the same medical diseases as the exposed patients (n. 229,766). They selected up to four unexposed individuals from each unexposed group to compare with an individual of the exposed group. They found that patients with a history of corticosteroid prescription had an increased risk of suicidal behaviours and neuropsychiatric disorders, mainly depression, mania and panic disorders. The distinction between corticosteroid effects and the severity of diseases is one of the main bias to overcome and in this study it was found that patients with asthma, polymyalgia rheumatica and giant cell arteritis who received corticosteroid treatments were at lower risk to develop neuropsychiatric disorders compared with patients with other diseases. The authors' hypothesis for the lower incidence of neuropsychiatric disorders in asthma patients was that they might have been chronically exposed to low doses of inhaled corticosteroids in the past and this might have somehow protected them, but no other hypothesis was proposed for the other two conditions. Randomized placebo controlled studies should be performed in order to distinguish the impact of the type and severity of a disease from the effects of corticosteroid treatment in the onset of neuropsychiatric disorders, but the design of these studies would be ethically unacceptable (Fardet *et al.*, 2012). Gathering similar data in terms of diagnostic standardization and size from veterinary clinics is logistically impossible, but the use of corticosteroid drugs in veterinary practice has been investigated through a survey conducted of three UK veterinary practices and a wide variation in prescribing patterns was detected. It was found that 14.55% of dog consultations resulted in systemic corticosteroid therapy (O'Neill *et al.*,

2012). Despite this considerable percentage of prescriptions and considering the large amount of literature in other species that indicates the possible influence of corticosteroid drugs on behaviour, no retrospective analysis has ever been done to investigate possible relationships between corticosteroid therapies and behavioural problems in dogs. The retrospective analysis of cases presented in this chapter, for the first time, investigates the possible implications of history of treatment with corticosteroids in a population of dog behaviour patients. The data were collected from February 2012 to November 2014. A sample of 345 dogs between 1 and 10 years of age, presented for behavioural problems, was selected from the database of the author's professional caseload and analyzed. The goals were to gather information about the proportion of dogs with behaviour problems who had a history of corticosteroid therapies and to investigate possible relationships with behaviour problems and the affective states associated with these problems. The relationship between history of corticosteroid therapy and the prescription of psychoactive drugs was also investigated, because this might be related to the severity of behaviour problems (Dodman *et al.*, 1996; King *et al.*, 2000; Marder, 1991; Pineda *et al.*, 2014).

5.2 Material and Methods

5.2.1 Sample

The files of 345 dogs aged between 1 and 10 years that had presented for behavioural problems were selected from among the dog caseload of the author's own veterinary behavioural clinic based in the north of Italy. This age range was selected to decrease the probability that reported behaviour problems were due to management problems in puppies or health conditions due to aging in older dogs. The data were collected from February 2012 to November 2014.

5.2.2 Investigated items

Each case file was analyzed and the following items of data were extracted for the purpose of this investigation:

1. Age of dog;
2. Gender and reproductive status of dog;

3. Breed type divided into the number of Federation Cinologique International Group (FCI: see Appendix F) for all the recognized breeds. Pitbull type dogs were coded as “Pitbull”, with crossbreed dogs coded as “crossbreed”;
4. Treatment and medical history of dogs treated with corticosteroid drugs;
5. Treatment and medical history of dogs treated with other drugs;
6. Presence of aggression towards people;
7. Types of bites (severe or not severe);
8. Behaviour problems other than aggression;
9. Prescription of psychoactive drugs to address the behavioural complaint;
10. Affective state (see below for details about definition of positive or negative affective state).

The source of the above information was both the Dog Behavioural History Form (See Appendix D) that was completed for every dog at the time of the first consultation, and the therapist’s own diagnostic and therapeutic notes.

For “history of treatment with corticosteroids” only systemic treatments that lasted more than one week were taken into consideration, in order to avoid single treatments for emergency interventions, since the effects of longer term administration were the focus of interest. Data about treatment with corticosteroid drugs was extrapolated from the Dog Behavioural History Form completed during behavioural consultation in the section concerning the medical history of patient. The exact administered dose was not always recalled by owners and an inclusion requirement for those subjects recorded as “received treatment with corticosteroids” was that the owner could recall the name of the product, the reason why it was prescribed and the length of the therapy in terms of more than a week or less than a week. As far as the item “history of treatment with other drugs” (Item 5) was concerned, reported treatments were considered as for treatment with corticosteroids.

As far as the item “types of bites”, bites that required medical intervention were considered as “severe bites”. Negative and positive affective states (Item 10) were coded (see below) on the basis of the responses of the owners to questions included in the Dog Behavioural History Form (Appendix D) specifically in the section entitled “Your

dog's personality" in conjunction with the evaluation of the veterinary behaviourist who held the consultation. As far as the information extracted from the Dog Behavioural History Form was concerned, owners had to answer "yes" or "no" to the following questions:

1. Do you consider your dog as aggressive in most situations?
2. Do you think that your dog is often or always nervous or fearful in the presence of unknown situations or stimuli (sounds, new stimuli, unknown people or dogs)?
3. Do you consider your dog largely enthusiastic and excited?
4. Do you think that your dog is sociable in general?
5. Do you consider your dog confident?

To be coded as having a positive affective state, the owner had to answer "No" to questions one and two and "Yes" to at least two of the last three questions. To be coded as having a negative affective states the owner had to answer "Yes" to at least one of the first two questions. The final definition of affective state as positive or negative in connection with the type of behaviour problem was established through the behavioural consultation and based on the description of the problem behaviour and its context as well as the direct observation of the dog's behaviour in the clinical context by the behaviour clinician.

Positive affective state was attributed to dogs with problem behaviours that were most likely to be related to positive emotions but perceived as problematic and/or exaggerated by their owners, for example:

- Excessive play behaviour;
- Excessive activity such as running, digging, stealing objects;
- Excessive attention seeking behaviours, such as jumping on people, barking for attention.

Negative affective state was attributed to dogs presenting with behaviours that were more likely to be related to negative emotions such as fear, anxiety and frustration. For example:

- Fear, phobia and anxiety;

- Avoidant or assertive displays of aggressive behaviours;
- Repetitive conflict behaviours.

5.2.3 Statistics

Analysis was performed using SPSS 21, with summary descriptive statistics calculated initially.

Possible relationships between dogs that received treatment with corticosteroids versus dogs that received a treatment with other drugs different from corticosteroids and:

- Positive or negative affective states;
- Aggression towards people;
- Other complained about behaviours different from aggression;
- Occurrence of severe bites;
- Prescription of psychoactive drugs

were assessed using a Pearson's Chi Squared test.

Regression analysis was then performed in order to hierarchically evaluate the best predictors of the investigated items ($p < 0.05$).

5.3 Results

From the initial sample of 345 dogs selected from the author's caseload database containing completely filled Dog Behavioural Forms, 2 dogs were excluded because of incomplete reports about medical history and treatment and a final sample of 343 was used for analysis.

Gender and reproductive state of dogs are shown in Table 5.1.

Gender and reproductive state	Frequency n.(%)		
	Total	No cortico group	On cortico group
entire male	177 (51.6)	147 (51)	30 (54.5)
entire female	56 (16.3)	49 (17)	7 (12.7)
castrated male	35 (10.2)	29 (10.1)	6 (10.9)
spayed female	75 (21.9)	63 (21.9)	12 (21.8)

No cortico group = group of dogs without a history of corticosteroid treatment;
On cortico group = group of dogs with a history of corticosteroid treatment.

Table 5.1: Gender and reproductive state distribution within the sample of 343 dogs.

Within the sample of 343 dogs, the age distribution of dogs is shown in Table 5.2.

Age	Frequency n.(%)		
	Total	No cortico group	On cortico group
1-2 years	195 (56.9)	179 (62.2)	16 (29.1)
3-4 years	87 (25.4)	70 (24.3)	17 (30.9)
5-6 years	34 (9.3)	24 (8.3)	8 (14.5)
7-8 years	26 (7.6)	14 (4.9)	12 (21.8)
8-9 years	3 (0.9)	1 (0.3)	2 (3.6)

No cortico group = group of dogs without a history of corticosteroid treatment;
On cortico group = group of dogs with a history of corticosteroid treatment.

Table 5.2: Age distribution within the sample of 343 dogs.

Breed distribution within the sample of 343 dogs is shown in Table 5.3:

Breed/type group	Frequency n. (%)		
	Total	No cortico group	On cortico group
FCI Group 1 Sheepdogs and Cattledogs	45 (13.1)	34 (11.8)	11 (20.0)
FCI Group 2 Pinscher and Schnauzer/ Molosoid and Swiss Mountain and Cattledogs	43 (12.5)	32 (11.1)	11 (20.0)
FCI Group 3 Terrier	23 (6.7)	17 (5.9)	6 (10.9)
FCI Group 4 Dachshunds	8 (2.3)	6 (2.1)	2 (3.6)
FCI Group 5 Spitz and primitive types	10 (2.9)	10 (3.5)	0
FCI Group 6 Scent hounds and related breeds	8 (2.3)	8 (2.8)	0
FCI Group 7 Pointing Dogs	9 (2.6)	8 (2.8)	1 (1.8)
FCI Group 8 Retrievers/Flushing Dogs/Water Dogs	18 (5.2)	13 (4.5)	5 (9.1)
FCI Group 9 Companion and Toy Dogs	29 (8.5)	25 (8.7)	4 (7.3)
FCI Group 10 Sighthounds	7 (2.0)	7 (2.4)	0
Crossbreed	126 (36.7)	113 (39.2)	13 (23.6)

Table 5.3: Continued on next page

Breed/type group	Frequency n. (%)		
	Total	No cortico group	On cortico group
Pitbull	17 (5.0)	15 (5.2)	2 (3.6)

No cortico group = group of dogs without a history of corticosteroid treatment;

On cortico group = group of dogs with a history of corticosteroid treatment.

Table 5.3: Breed distribution within the sample of 343 dogs. (For description of FCI groups see also Appendix F).

Within the sample of 343 dogs, 209 (73.8%) had a history of treatment with drugs other than corticosteroids and 55 (16.0%) had a history of treatment with corticosteroid drugs. Forty-eight of the 55 dogs that had a history of treatment with corticosteroids also had a history of treatment with other drugs. In Table 5.4, the types of drug treatments other than corticosteroid in the two groups of dogs with and without a history of treatment with corticosteroid drugs are shown.

Drug therapies have been divided into four main categories:

- Antibiotics and antifungal drugs;
- Antacid drugs;
- Non Steroidal Anti-inflammatory drugs (NSAIDs);
- Other drugs.

TYPE OF DRUG	Frequency n.(%)	
	No cortico group (n.209)	On cortico group (n.55)
antibiotics and antifungal drugs	162 (78.3)	32 (66.7)
antacid drugs	18 (8.7)	12 (25.0)
NSAIDS	17 (8.2)	3 (6.3)
Others	10 (4.8)	1 (2.1)

No cortico group = group of dogs without a history of corticosteroid treatment;

On cortico group = group of dogs with a history of corticosteroid treatment.

Table 5.4: Types of drug treatments different from corticosteroids in the two groups of dogs.

Reported medical problems were divided into 5 main groups:

- Dermatological;
- Orthopaedic;
- Gastroenteric;
- Respiratory;
- Others.

In “Others” were included urinary and reproductive problems, cardiovascular and hematological diseases and neurological diseases.

Medical problems reported in dogs treated with corticosteroid drugs and dogs treated with drugs other than corticosteroids are show in table 5.5.

Medical Problems	Frequency n.(%)	
	No cortico group	On cortico group
Dermatological	53 (18.4)	36 (65.5)
Orthopaedic	15 (5.2)	12 (21.8)
Gastrointestinal	74 (25.7)	5 (9.1)
Respiratory	54 (18.8)	1 (1.8)
Others	33 (11.5)	1 (1.8)
No medical problems with more than 1 week therapy	79 (27.2)	0

No cortico group = group of dogs without a history of corticosteroid treatment;

On cortico group = group of dogs with a history of corticosteroid treatment.

Table 5.5: Medical problems in dogs a history of treatment with corticosteroids. The sum of percentages is not 100% because of multiple medical problems.

Within the sample of 343 dogs the main behavioural complaints are listed in Table 5.6. Specific conditions had to be pooled into broader categories as described below. All forms of aggressive behaviour towards people with the exception of that associated with play, were pooled.

Aggression towards people included both aggression towards family members and aggression towards strangers.

Aggression towards other dogs included both aggression between dogs living together in the same family and dogs that were aggressive towards unfamiliar dogs.

Predation (“predatory aggression”) was complained about for just one dog and it was referred for severe predatory behaviour towards cats.

Separation problems refers to all problem behaviours that happened in the absence of owners/when the dog was left alone that were associated with distress.

House-soiling problems includes both urine marking and forms of inappropriate elimination and housetraining problems.

Phobias refers to fear of specific situations (e.g. thunderstorm, wind, unknown environments), social fear (fear of people, fear of other dogs) and fear of sounds.

Repetitive behaviours includes compulsive licking, tail chasing and tail biting.

Excessive barking refers to problematic barking in different situations.

Hyperactivity includes excessive play and play biting, excessive attention seeking behaviours, jumping on people, destructiveness and excessive excitement in different situations.

Other behaviour problems includes cases involving a tendency to run away from the property, digging and decreased activity levels.

Aggression towards people was the most common complaint relating to 43.1% of dogs (148). Multiple behaviour problems were presented in 66.5% of the total sample of dogs (228). Within the sample of dogs in treatment with corticosteroids, 51% presented multiple behaviour problems (28). Results are shown in Table 5.6.

Behaviour	Total frequency n. (%)
Aggression towards people	148 (43.1)
Aggression towards other dogs	59 (17.2)
Predation	1 (0.3)
Separation problems	47 (13.7)
Housoiling problems	22 (6.4)
Phobias	91 (26.2)
Repetitive behaviours	11 (3.2)
Excessive barking	43 (12.5)
Hyperactivity	90 (25.9)
Other	37 (10.7)

Table 5.6: Categories of presenting complaint; the sum of percentages is not 100% because of multiple behaviour problems.

Psychoactive drugs were prescribed to 31.2% of the sample (107 dogs). Among the 55 dogs with a history of corticosteroid treatments psychoactive drugs were prescribed in the 43.6% of cases (24 dogs). Among the 290 dogs without any history of corticosteroid drugs psychoactive drugs were prescribed in the 28.4% of cases (83 dogs).

The distribution of drugs used is shown in Table 5.7.

Prescribed drug	Frequency n.(%)		
	Total	No cortico group	On cortico group
Fluoxetine	69 (20.1)	50 (17.4)	19 (34.5)
Fluvoxamine	9 (2.6)	9 (3.1)	–
Paroxetine	11 (3.2)	10 (3.5)	1 (1.8)
Selegiline	9 (2.6)	8 (2.8)	1 (1.8)
Clomipramine	8 (2.3)	5 (1.7)	3 (5.5)
Carbamazepine	1 (0.3)	1 (0.3)	–

No cortico group= dogs that did not have an history of treatment with corticosteroid drugs.

On cortico group = dogs had a history of treatment with corticosteroid drugs.

Table 5.7: Psychoactive drugs prescribed within the whole sample (n = 343 dogs).

The possible relationships between corticosteroid therapies, behaviour problems,

affective states, incidence of severe bites and prescription of psychoactive drugs are shown in Table 5.8. These relationships were also investigated taking into account only dogs that had a history of medical problems that required more than one week of therapy ($n = 264$) using Chi Square Tests.

Within this sample ($n = 264$), dogs with a history of corticosteroids were significantly more likely to be in negative affective states ($X(1) = 10.970$, $p < 0.001$), to present aggression towards people ($X(1) = 5.527$, $p = 0.0014$) to be prescribed psychoactive drugs with ($X(1) = 3.588$, $p = 0.043$), but significantly less likely to present problems coded as “hyperactivity” ($X(1) = 9.099$, $p < 0.001$). See also table E.22 in Appendix E.

Patient Factors	n.(%)		Pearson Chi Square	p value
	No Cortico group	On Cortico group		
Positive affective state	51 (17.7)	0		
Negative affective state	237 (82.3)	55 (100.0)	11.441	< 0.001
Aggression towards people	117 (40.6)	31 (56.4)	4.663	0.023
Hyperactivity	85 (29.5)	4 (7.3)	11.889	< 0.001
Aggression towards other dogs	50 (17.4)	9 (16.4)	0.032	0.518
Separation problems	38 (13.2)	9 (16.4)	0.330	0.392
Phobias	77 (26.7)	13 (23.6)	0.229	0.384
Excessive barking	38 (13.2)	5 (9.1)	0.709	0.276
History of severe bites	64 (22.2)	16 (29.1)	1.218	0.175
Psychoactive drug prescription	83 (28.8)	24 (43.6)	4.724	0.024

No cortico group = group of dogs without a history of corticosteroid treatment;

On cortico group = group of dogs with a history of corticosteroid treatment.

Table 5.8: Relationship between history of treatment with corticosteroids and negative affective state, reported behaviour problems, occurrence of sever bites and the prescription of psychoactive drugs in the whole sample ($n. 343$).

The predictive value of age, gender and type of medical problems in the onset of

positive or negative affective states was investigated in the group of dogs without a history of corticosteroid drugs but with a history of medical problems ($n = 209$) by means of binary logistic regression using forward analysis. Age was found to be the only predictor, with dogs between one and two year of age 3.167 times more likely to be in positive affective states than other ages ($B = 1.153$; $p < 0.01$; $\exp(B) = 3.167$; C.I: 95% [1.44 - 6.96]). The percent of cases for which affective state was correctly predicted was 82.8.

The role of corticosteroid treatment history, medical condition, age and gender of dogs on the main effects of aggression towards people, hyperactivity and prescription of psychoactive drugs were investigated by means of binary logistic regression using forward analysis .

The best predictor for aggression towards people was gender, with males 3.49 times more likely to be aggressive compared with gender other than male ($B = 1,250$; $\exp(B) = 3.489$; $p < 0.01$; C.I. : 95% [2.10 - 5.79]) and castrated male dogs 2.8 times more likely to show aggression compared with gender other than castrated male ($B = 1.049$; $\exp(B) = 2.828$; $p = 0.011$; C.I. : 95% [1.27 - 6.28]). Age and gastrointestinal conditions showed a significant relationship with aggression towards people: dogs between one and two years were less likely to be aggressive towards people ($B = -0.752$; $\exp(B) = 0.471$; $p < 0.01$; C.I. : 95% [0.312 - 0.802]) as were dogs with history of gastrointestinal conditions ($B = -0.809$; $\exp(B) = 0.445$; $p < 0.01$; C.I. : 95% [0.250 - 0.792]). The percent of cases for which the aggression towards people was correctly predicted was 64.7.

Best predictors for hyperactivity were age and corticosteroid treatment: dogs between one and two year of age were 4.27 times more prone to show hyperactivity compare with other ages ($B = 1.193$; $\exp(B) = 3.298$; $p < 0.01$; C.I. : 95% [1.78 - 6.12]). By contrast, dogs treated with corticosteroids were less prone to hyperactivity ($B = -1.455$; $\exp(B) = 0.233$; $p < 0.01$; C.I. : 95% [0.08 - 0.68]). The percent of cases for which hyperactivity was correctly predicted was 74.1.

Best predictor for the prescription of psychoactive drugs were gender and age: castrated males were 2.49 times more likely to be prescribed psychoactive drugs compared with gender other than castrated male ($B = 0.911$; $\exp(B) = 2.486$; $p = 0.014$; C.I.: 95% [1.20 - 5.14]). Dogs between one and two years of age were less likely to receive prescriptions for psychoactive drugs compared with other ages ($B = -0.730$; $\exp(B) = 0.482$; $p < 0.01$; C.I. : 95% [0.30 - 0.77]). The percent of cases for which prescription

of psychoactive drugs was correctly predicted was 70.6.

5.4 Discussion

The purpose of this final study was to examine the evidence of the effects of corticosteroids in a clinical context with a view to highlighting the importance of giving correct behavioural advice to owners when corticosteroids have to be prescribed. This work, on its own, is not sufficient to demonstrate causal links between corticosteroid treatment and a particular behavioural problem.

Behavioural diagnoses are often difficult to define and many dogs with behavioural disturbances are diagnosed with multiple issues, many of which are linked to anxiety, or a negative cognitive bias (Adams and Clark, 1989; Wells and Hepper, 2000).

Within the sample of 343 dogs referred for behaviour problems in this particular case-load, 16% had a history of previous treatments with corticosteroids.

The main finding of this retrospective case study was that history of corticosteroid treatment is significantly related with negative affective states and is a negative predictor for problems of hyperactivity.

Age was another significant predictor for affective states with dogs between one-two year of age being more probably in positive affective states than older dogs. Age was also a significant predictor for hyperactivity and younger dogs were more likely to be presented for hyperactivity problems. Again, younger dogs were less likely to show aggression towards people and to be the target for the prescription of psychoactive drugs. On the contrary, male dogs were more likely to show aggression towards people and this result is consistent with some of the literature data (Fatjo *et al.*, 2007; Guy *et al.*, 2001; Overall and Love, 2011; Overall, 1997).

The history of medical problems was not a significant predictor for behaviour problems with the exception of gastrointestinal problems that was linked with a lower tendency to show aggression towards people.

These results must be cautiously considered, but they provide a useful contribution to the picture of possible behavioural side effects of corticosteroids and a starting point for future investigation.

The small sample size and the differences in the two groups, with and without history of corticosteroid drug treatments, are obvious limitations, but it is worth noting that medical history was not revealed to have a strong effect in this study. Medical

problems and ages in the two groups are very different: in the group of dogs with a history of corticosteroid treatment dermatological and orthopedic problems are over-represented compared with the other group which is perhaps not surprising given the indications for these drugs; gastrointestinal conditions are most numerous in the group of dogs without a history of corticosteroid drugs and perhaps unsurprisingly least numerous in the other group, since they would rarely be indicated for such problems. Most dogs in the “no history of corticosteroid treatment” group were less than two years of age, while this age range was much less represented in the “history of corticosteroid treatment” group. The effects of medical histories cannot be reliably evaluated here due to the low number of cases and although the effect of pain and discomfort on dog behaviour is an important issue (Barcelos *et al.*, 2015; Camps *et al.*, 2015) and medical problems are important sources of distress (Mills *et al.*, 2014; Notari, 2009), no medical problem was a reliable predictor for negative affective states. As stated above, the main finding of this investigation was that dogs presented for behaviour problems with a history of corticosteroids treatment appear to suffer more from problems associated with negative affect. The finding of a significant relationship between negative affect and corticosteroid treatment suggests that exposure to corticosteroids might increase dogs’ sensitivity to environmental and social stressors increasing the risk of problem behaviour as a consequence compared with dogs without a history of corticosteroid treatment. Given that the sample of dogs with a history of corticosteroid drugs was quite small (55 dogs), random effects have a greater chance of biasing the results, and so this study should not be considered definitive. Dermatological conditions were over-represented in the group of dogs with a history of corticosteroid drugs (65.5%), compared with the other group of dogs (18.4%) and, even though dermatological condition was not found to be a predictor for affective state, a combined effect of drugs and disease on negative affective states cannot be excluded. A matched control study would provide stronger evidence.

It has been shown that pruritus might be exacerbated by psychological disorders in humans but also that pruritus can worsen behaviour (Shaw *et al.*, 2007). The issue of the influence of pruritus as a main sign of dermatological conditions was investigated by Klink *et al.* and they found no relationship between pruritus and aggression or anxiety, but they did find a significant increase in reactivity to potentially fearful stimuli in dogs treated with corticosteroid drugs (Klink *et al.*, 2008). This reported increase in reactivity to negative stimuli seems to be consistent with our finding of an

increase in negative affective states due to corticosteroid treatment.

Corticosteroids and age were significant predictors for problems of hyperactivity. Dogs with a history of corticosteroid treatment were significantly less likely to be hyperactive, and, given the definition of hyperactivity used in this study, this result might be consistent with the decreased tendency to play shown in the survey described in Chapter 3. Furthermore, in the preliminary survey (Chapter 2) two owners of dogs that received treatments with corticosteroids reported a decrease in their dogs' activity levels. The item "Hyperactivity" included a list of problematic behaviours like play and play biting, excessive attention seeking behaviours, jumping on people, destructiveness, excessive excitement in different situations, all of which might be considered to reflect positive affect (reward seeking). The term did not imply a diagnosis of pathological hyperactivity associated with impaired attention as in human beings (Blum *et al.*, 2008; Wright *et al.*, 2012).

In the caseload presented here, 16% of dogs had a history of treatment with corticosteroid drugs. A slightly lower percentage of corticosteroid prescriptions in pet dogs was reported in general veterinary practice for different diagnosis (14.55%) in the study of O'Neil *et al.* (O'Neill *et al.*, 2012). In dermatological conditions 20% of dogs have been reported to receive a prescription of corticosteroid drugs (Hill *et al.*, 2006) and in our caseload the percentage of dogs with a history of dermatological conditions, corticosteroid drug prescription was more common (40.4%). However, as there was not a control group from general veterinary practice included in this study, it was not possible to establish if dogs treated with corticosteroids were over represented among subjects with behavioural problems, although these percentages indicate that the issue is an important one.

The results concerning affective states are consistent with previous findings and it is now accepted that animals can feel a range of negative and positive emotions (Boissy *et al.*, 2007; Reimert *et al.*, 2013). More and more attention has been given to the issue of how affective states of animals may influence behaviour and learning (Bentosela *et al.*, 2009; Burman *et al.*, 2011), but their assessment in animals is still difficult to define, mainly because they rise from subjective experience. In human beings this assessment relies mostly on verbal communication while in animals it has to rely on the observation of the individual, assuming that animal behaviour is the result of the acquisition and assessment of environmental information and the elaboration of an observable response. It has been shown that fearful and anxious animals tend to show

behaviours that can be interpreted as motivated by a decrease in their anticipation of positive events when presented with ambiguous stimuli. This kind of “pessimistic” perspective has been called a negative “cognitive bias” or “judgement bias” (Paul *et al.*, 2005). Laboratory animals housed in deprived or unpredictable environments showed a judgment bias and were less likely to anticipate a reward – and act consequently – when presented with ambiguous stimuli (Boissy *et al.*, 2007; Burman *et al.*, 2011; Mellor, 2012; Starling *et al.*, 2013). It has also been shown that dogs with separation anxiety expressed a pessimistic judgment bias that resolves with treatment (Karagiannis *et al.*, 2015; Mendl *et al.*, 2010). In this retrospective study, no specific behaviour problem was found to be linked with a history of corticosteroid drugs while in the surveys carried out in the previous part of this research (see Chapter 2 and 3), owners reported behavioural changes in specific areas of their dogs’ behaviour.

Nevertheless these results deserve attention because a higher tendency to be in negative affective states might be particularly important for individual dogs that already have the tendency to show avoidance or aggressive behaviours.

Systemic corticosteroid prescription in pet dogs seems to be determined more by the clinical experience and personal opinion of vets (O’Neill *et al.*, 2012) and greater recognition of the widespread risk of possible drawbacks from these drugs, including the conditions identified in this research, might contribute to improved evidence-based guidance for the therapeutic use of these drugs to minimize these risks (see the “Check list for veterinary practitioners” in Appendix F).

5.5 Conclusion

The present chapter has been introduced in order to provide a clinical context to the preceding studies and to emphasize the importance of considering the issue of possible behavioural effects of corticosteroid treatments.

The data from this work, along with the results of the surveys and the behavioural tests, strongly suggest that advice should be given when these drugs are prescribed, in particular when dogs show or have shown behaviour problems.

Chapter 6

General Discussion And Future Directions

Behavioural adverse reactions to corticosteroid treatment in dogs were apparent in each of the studies in this thesis. Exploring the effects on behaviour of important players in stress responses such as corticosteroids was and is not an easy task even in human medicine. In the human field there are a large number of case reports and studies reported on the possible psychiatric side effects of these drugs, but no similar data have been available in veterinary medicine, prior to the onset of this work.

The first investigation, described in Chapter two, was an attempt to discover what kind of behaviours might change under corticosteroid therapy in dogs, and owners reported several changes that were consistent with the literature. The more structured survey that was carried out building on the results of the first preliminary investigation (Chapter three), reported similar changes in the behaviour of dogs receiving corticosteroid therapy. In fact, the answers to the questionnaire were impressively consistent with the answers given by owners in the preliminary study but the discovery of a significant reduction in play behaviour in dogs treated with corticosteroids suggested that a more general increase in negative affective bias might have been behind the behaviour changes observed. The behavioural test results, presented in Chapter four, showed that dogs in treatment with corticosteroids were less exploratory after the growl: this behavioural inhibition was interpreted as a sign of increased sensitivity to the mild anxiety provoking stimulus that was proposed. Finally, the behavioural caseload in Chapter five showed that dogs with a history of corticosteroid drugs were more likely to be in negative affective states and less likely to be hyperactive for reasons associated with positive affective state compared with dogs that did not received these drugs in the past.

An increased sensitivity to negative stimuli is likely to affect the welfare of dogs and might also explain why dogs on corticosteroids were reported to show more avoidance behaviours and even aggression.

Both in humans and non-human animals, normal stress responses, when appropriate, should increase adaptation and increase the chances of survival but extremely intense, unpredictable repeated, chronic stress may become maladaptive and cause severe affective problems. These problems have a common denominator: a negative affective state.

Changes in behaviour related to negative affects reflect changes in the brain areas involving emotion and cognition and there is evidence that not only stressful conditions but also exogenous corticosteroids might damage brain structures that are crucial for

memory and emotions. For example damage in the hippocampus has been demonstrated in rodents and in primates (Sapolsky *et al.*, 1990) and also hypothesized in humans using MRI (Höschl and Hajek, 2001). The hippocampus is deeply involved in short-term memory consolidation and HPA axis regulation and damage, such as atrophy, might be induced by corticosteroid drugs (Brown *et al.*, 1999; Höschl and Hajek, 2001). Many factors have been recognized as a risk for increasing hippocampal vulnerability to corticosteroids and it was described that both chronic stress and prolonged exposure to corticosteroids can cause hippocampal dendritic retraction but not irreversible cell death. This dendritic retraction might increase hippocampus vulnerability to metabolic changes or neurotoxins. Hippocampal dendritic retraction has been related with spatial memory deficits, contextual fear conditioning and also depression-like behaviours (Qiao *et al.*, 2016; Conrad, 2008). This has been called the “glucocorticoid vulnerability hypothesis” (Conrad, 2008). Brown *et al.* used MRI to detect reduced amygdala volume in human patients treated with corticosteroids (Brown *et al.*, 2008). The right amygdala volume was inversely related to the length of corticosteroid therapy. This study suggests that the amygdala, as well as the hippocampus, is sensitive to corticosteroid effects but, beyond this conclusion, the interpretation of these results is not easy.

As already mentioned in the previous part of this thesis, the amygdala is a brain structure deeply involved in emotion, particularly fear, and plays an important role in stress responses. It has been shown that amygdala volume was smaller in humans suffering from Post-Traumatic Stress Disorders compared with non-pathological controls, and this was also associated with a smaller left hippocampal volume (Morey *et al.*, 2012). By contrast, Lupien *et al.* investigated hippocampus and amygdala MRI images in children suffering from depression due to early separation from their mothers (Maternal Distress Syndrome) and found larger amygdala volumes in affected children compared with non-affected controls (Lupien *et al.*, 2011). The different effects of exogenous corticosteroids on neural cells have not been completely clarified and synthetic corticosteroids modulate neural cells and might produce detrimental or positive effects that appear to be mediated by dose, individual features, and brain region phenomena (Numakawa *et al.*, 2010; Ramos-Remus *et al.*, 2002). Vyas *et al.* described different patterns of modification of the volume of hippocampus and amygdala in rats following chronic immobilization stress with dendritic atrophy in the hippocampus but increased arborization in the neurons of basolateral complex of the

amygdala (Vyas *et al.*, 2002; see also Chapter 1). Investigating similar neuroanatomical changes in dogs is beyond the scope of this thesis, although neuroimaging is increasingly used in dogs and studies that investigated dog brain areas related to the perception of environmental and social stimuli have recently been carried out even in conscious dogs (Andics *et al.*, 2015; Berns *et al.*, 2014). Neuroimaging studies have been done to investigate the role of serotonin in impulsive aggression in dogs (Peremans *et al.*, 2003) and also to study the influence of ketamine on cerebral blood perfusion and on serotonin receptors in the canine brain (Waelbers *et al.*, 2015). The effects of aging on dog brain was also investigated through MRI in beagle dogs and several areas of the brain, included hippocampus, were investigated and measured (Kimotsuki *et al.*, 2005), so there is no theoretical barrier to undertaking such work in dogs. The use of advanced neuroimaging techniques to investigate brain area that are crucial for cognition and emotion in dogs might provide important information about the impact of corticosteroid drugs on brain structures such as amygdala and hippocampus, as has already been done in human beings.

Merging the results of brain imaging examinations and behavioural tests might provide a more complete picture of the effects of corticosteroid drugs on dog brain and behaviour. Although these kinds of investigations are challenging from economic, practical and potentially ethical perspectives, their outcomes are likely to provide important information.

Behavioural tests remain an important tool to objectively assess behaviour and emotional states and in future studies the use of a cognitive bias paradigm in order to investigate the effects of corticosteroid drugs in dogs might be a useful tool to evaluate possible shifts in cognitive bias as illustrated by Karagiannis *et al.* (Karagiannis *et al.*, 2015). A negative bias is explained as a tendency to exhibit more intense behavioural and physiological responses to negative stimuli and less intense responses to positive stimuli, resulting in a more “pessimistic” appraisal of social and life situations. It has been shown that corticosteroids and norepinephrine interactions have an important role in the emergence of negative bias (Kukolja *et al.*, 2008).

Future directions in this field should consider some of the issues that have not been addressed in this research work. One of the main questions that should be addressed is the proportion of dog patients that show behavioural side effects after administration of GCs drugs, in order to better quantify the risk.

Another important issue that needs to be further investigated is the relationship

between the dose of GCs and the onset of behavioural changes. The corticosteroid doses recorded throughout this research work were mostly around the low therapeutic range of doses and our sample was targeted mainly towards patients with moderate rather than severe medical conditions, in order to minimize the effect of pain and physical impairment. In human patients, investigations into effects of corticosteroid drugs have also been done in patients with severe disease such as nephrotic syndrome. These studies have shown that high-dose corticosteroid treatment produces important behavioural changes including aggression (Hall *et al.*, 2003; Mishra *et al.*, 2010). The available literature data in human medicine report that the psychiatric symptoms during corticosteroid therapy are dose dependent and often occur early in treatment. It was also shown that these effects are reversible with discontinuation of the therapy (Brown and Chandler, 2001), and this is another issue that would be important to investigate in dogs.

Because it was reported that in patients with a history of neuropsychiatric disorders multiple treatment courses with corticosteroids was associated with the risk of having the same disorders in subsequent corticosteroid treatments (Fardet *et al.*, 2012), it would also be worth investigating whether multiple treatment with corticosteroids in dogs might be associated with the recurrence of behaviour problems.

Sensitivity to chemicals and drugs is related to individual features and individual differences in endocrine reaction have been shown in genetically selected laboratory animals (Liebsch *et al.*, 1998). Genotype and early experiences have been shown to influence stress-reaction patterns and also reaction to exogenous corticosteroids (Mirescu *et al.*, 2004). Corticosteroid sensitivity might depend on the different distribution of GRs and MRs and the regulation of their action in the brain, which are profoundly influenced by genetic factors (De Kloet and Derijk, 2004; De Kloet *et al.*, 1996). The issue of genetic influence on stress and corticosteroid sensitivity in dogs is an important one and investigating different changes in behaviour as a consequence of corticosteroid therapies in different breeds will also provide important information about the impact of stress on dog behaviour.

Starting from this first research the proposed check-sheet for veterinary practitioners mentioned above (Appendix G) might be broadly distributed in order to produce a large volume of data and provide more information about the impact on behaviour of corticosteroid dosage, length of treatment, reason for treatment and individual treated dog features including their gender, age and breed. Significant results of such a survey

will also be likely to facilitate the recruitment of a larger sample of dogs undergoing treatment with corticosteroid drugs for behavioural tests because veterinarians might be more aware of the importance of this issue and, as a consequence, more motivated to recruit dogs. The behavioural tests described in this research might be replicated with a larger sample and/or other behavioural tests might be introduced. As mentioned above a cognitive bias paradigm might be used to assess affective states, as already suggested by Burman (Burman, 2014), using test designs that have already been used in other studies (see for example Karagiannis *et al.*, 2015).

The behaviour of dogs is influenced by many different factors and an important one is the dog's perception of human behaviour; since it was shown that dogs can understand human gestures and gaze (Cunningham and Ramos, 2014; Topál *et al.*, 2014), behavioural test involving responses to human communicative cues might be used as a possible instrument to detect possible changes in social responses when dogs are in treatment with corticosteroid drugs.

Throughout this research we have tried to systematically investigate an unexplored field that has multiple implications for dog behaviour and welfare as well as for dog-human relationships. Companion dogs are more than just "pets" and many studies have shown the importance of the dog-human bond in enhancing human well-being (Hart, 1995; Siegel, 1990). Dog-owner relationships might have several dimensions: a dog can be perceived as a relative, a friend or might represent a status symbol or an extension of self (Dotson and Hyatt, 2008; Veevers, 2008). Negative changes in companion dog behaviour will often have a deep impact on the life of its carers and investigating behavioural responses to human cues in dogs treated with corticosteroids might provide further tools to prevent detrimental effects on dog-owner relationships due to change in dogs' behaviour as a consequence of corticosteroid treatments used as part of the normal healthcare of the individual.

Important advances in our knowledge have been made with practical implications for better practice, alongside suggestions for future research directions. Behavioural medicine is a field that is becoming increasingly important within the veterinary profession and the results presented here provide a small, but significant, piece in the puzzle of understanding the link between corticosteroid drugs and dog behaviour: other pieces should be added in the future.

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Appendix A

PRESS RELEASE UK



Press release

Anglo-Italian research launched into drug side effects in dogs

Researchers in Lincoln and Milan have launched a new study into the behavioural side effects of drugs in dogs.

Many drugs have been adapted for safe use in animals in so much as they do not cause overt harm, but some are known to have psychological side effects, which until now have been largely unexplored.

Dr Lorella Notari, a veterinary surgeon in Milan, Italy, who is working towards a PhD at the University of Lincoln, is leading a new research project which will examine the behavioural changes in dogs being treated with certain medications.

The researchers are particularly interested in the side effects of a group of commonly used anti-inflammatory drugs known as corticosteroid or ‘steroids’. These are often used in conditions like chronic skin problems or arthritis.

The researchers have found initial evidence to suggest they may directly affect a number of behaviours related to dogs’ perception of others and their environment.

The team are therefore following up this initial research by launching an online questionnaire aimed at collating reports from owners of dogs with these medical conditions.

Professor Daniel Mills, from the University of Lincoln’s Department of Biological Sciences, said: “The aim of the survey is to recruit a large number of pet owners whose dog is currently receiving treatment for some form of arthritis or skin problem. We need information from owners of dogs using different treatments in order to examine if one drug is associated with specific changes. By asking owners to report on a range of indirect behavioural responses that they observe when their dog is on treatment, we can then calculate the chance that a certain drug is associated with a higher risk of certain changes in behaviour and whether this fits with our predictions and initial evidence. Later we will test this association with carefully designed behaviour tests in volunteered subjects.”

Dog owners who would like to be involved in the research can visit <http://www.dog-behaviour.org> where the questionnaire is available in both English and Italian.

For general press enquiries contact:

Ian Richards: Media and PR officer

(01522) 886042 irichards@lincoln.ac.uk

Visit our news web pages: www.lincoln.ac.uk/news/latestnews.htm

ENDS

PRESS RELEASE ITALY

Comunicato Stampa:

Nuovo studio sugli effetti comportamentali dei farmaci sui cani

É stato intrapreso un nuovo studio a Lincoln e Milano. Molti farmaci sono stati adattati per essere usati in sicurezza negli animali e non sono apparentemente rischiosi, ma alcuni possono avere degli effetti collaterali di tipo psicologico che finora non sono stati per nulla studiati. I ricercatori sono particolarmente interessati agli effetti collaterali di un gruppo di farmaci anti-infiammatori comunemente usati conosciuti come corticosteroidi o 'steroidi'. Questi farmaci sono spesso usati nei problemi cutanei o nell'artrite e i ricercatori hanno rilevato delle prime evidenze che suggeriscono che questi farmaci potrebbero anche influenzare direttamente diversi comportamenti legati alla percezione degli altri e dell'ambiente circostante. Essi stanno dunque perseguendo questa linea di ricerca, con il lancio di un questionario online che ha lo scopo di raccogliere informazioni dai proprietari di cani che hanno questi problemi medici. Il Professor Mills dell'Università di Lincoln spiega: "lo scopo dello studio è di raccogliere un grande numero di proprietari di cani che stanno attualmente assumendo terapie per alcune forme di artrite, artrosi o problemi dermatologici; abbiamo bisogno di informazioni su cani che seguono diverse terapie per esaminare se un certo farmaco è associato con cambiamenti specifici. Chiedendo ai proprietari di riferire una serie di risposte comportamentali che possono osservare quando il loro cane è in terapia, possiamo calcolare la possibilità che un certo farmaco sia associato con un rischio più alto di mostrare certi cambiamenti comportamentali e se questi risultati sono compatibili con le nostre ipotesi e con le prime evidenze da noi trovate. In uno stadio successivo testeremo questa associazione con test comportamentali appositamente studiati che verranno condotti con cani di proprietari che volontariamente si presteranno allo scopo".

Se desiderate partecipare, per favore andate all'indirizzo web

<http://www.dog-behaviour.org>

Dove è disponibile il questionario sia in Italiano che in Inglese.

FINE

QUESTIONNAIRE UK

OWNERS' QUESTIONNAIRE

This study is part of an International Research Project and we are very grateful for your assistance in completing it. Your personal details will remain confidential.

The goal of this questionnaire is to collect data about the possible effects of the medication that you are giving to your dog, that have not been reported before. These effects can be positive/favourable or negative/problematic for your dog and its management. This survey is about your dog's behaviour during the period of therapy. Your answers will be pooled with those of other dog owners and none of them will be directly attributed to you.

File n.

Date.....

NameTel.....e-mail.....

Dog name.....Breed.....Age.....M ☐ F ☐ Spayed/castrated ☐

Disease

Drug Dose (see note below).....

(Please, fill in the information about the drug including the instructions you have received from your vet: time of administration, change in dose in the following days or weeks etc. The dose can be expressed in milligrams or in tablets, specifying the dose for each tablet. For example if you give half a tablet you should specify the dose in milligrams in a tablet e.g 5 mg tablets half twice a day)

Other drugs taken in the same period (any treatment, in connection or not with the disease you mentioned above, for example antiparasitic drugs or insulin if your dog is diabetic)?

NO ☐ YES ☐

Drug Dose

Drug Dose

WHEN YOU ANSWER THE FOLLOWING QUESTIONS, YOU ARE KINDLY REQUESTED TO THINK, AT FIRST, ABOUT THE BEHAVIOUR OF YOUR DOG BEFORE STARTING THE DRUG AND PUT AN X WHERE APPROPRIATE IN THE UPPER UNSHADED STRIP. THEN YOU SHOULD THINK ABOUT YOUR DOG'S BEHAVIOUR WHILE YOUR DOG IS TAKING THE DRUG AND PUT AN X WHERE APPROPRIATE IN THE DARKER, LOWER STRIP

Q1. Play behaviour. Some dogs are very motivated to play with people, other dogs or toys. On a scale from 1 to 7 where 1 is 'not very playful' and 7 is 'very playful' how would you rate your dog's behaviour?

	Not very playful	←————→	————→	Very playful			
Without drug							
With drug							
	1	2	3	4	5	6	7

Q2. Nervousness/restlessness. Thinking about your dog's temperament, how would you define its nervousness/ restlessness on a scale from 1 to 7 where 1 is 'very nervous and restless' and 7 is 'very calm'?

	Very nervous/restless	←————→	————→	Very calm			
Without drug							
With drug							
	1	2	3	4	5	6	7

Appendix A

Q3. Insecurity and fearfulness. Thinking about your dog's general responses, for example, in the presence of unknown people or of new, unknown stimuli (sounds, loud voices, unknown contexts, unknown animals or children...), on a scale from 1 to 7 where 1 is 'extremely fearful and insecure' and 7 is 'very confident', how would you rate your dog?

Fearful/insecure ←————→ *very confident*

Without drug							
With drug							
	1	2	3	4	5	6	7

Q4. Food related aggression. Thinking about your dog's behaviour when there is food around, on a scale from 1 to 7 where 1 is not at all aggressive and 7 is very aggressive in the presence of food, how would you rate your dog?

Not at all aggressive over food ←————→ *Very aggressive over food*

Without drug							
With drug							
	1	2	3	4	5	6	7

Q5. Attention seeking behaviour. Some dogs tend to be very insistent and seek physical contact with owners by jumping up, snapping, scratching with a front paw, whining or barking: on a scale from 1 to 7 where 1 is 'no attention seeking behaviours' and 7 is 'frequent and intense attention seeking behaviours', how would you rate your dog?

No attention seeking ←————→ *Frequent & intense attention seeking*

Without drug							
With drug							
	1	2	3	4	5	6	7

Q6. Barking. Some dogs bark at any time, night and day, some others bark only in exceptional occasions. On a scale from 1 to 7 where 1 is 'rare barking' and 7 is 'frequent and intense barking', how would you rate your dog's behaviour?

Rare Barking ←————→ *Frequent and intense barking*

Without drug							
With drug							
	1	2	3	4	5	6	7

Q7. Obedience. Some dogs are very obedient, for example they come when called and go to bed when asked, while some others are less easily controlled. On a scale from 1 to 7 where 1 is 'not at all obedient' and 7 is 'very obedient', how would you rate your dog's behaviour?

Not at all obedient ←————→ *Very obedient*

Without drug							
With drug							
	1	2	3	4	5	6	7

Appendix A

Q8. Guarding behaviour. Some dogs are very predisposed to guarding behaviour and tend to threaten people by barking and growling, some others are friendly with everyone and don't show any guarding behaviour. On a scale from 1 to 7 where 1 is 'no guarding behaviour' and 7 is 'intense & frequent guarding behaviour', how would you define your dog's behaviour?

No guarding behaviour ←————→ Intense/frequent guarding

Without drug							
With drug							
	1	2	3	4	5	6	7

Q9. Startle reactions. Some dogs tend to startle very easy, for example when they hear a sound or are suddenly touched. In these cases they can react by fleeing, getting jumpy or showing aggression. On a scale from 1 to 7 where 1 is 'low/rare startle response' and 7 is an 'excessive and very frequent startle response', how would you define your dog's behaviour?

Low/rare startle responses ←————→ excessive/very frequent startle responses

Without drug							
With drug							
	1	2	3	4	5	6	7

Q10. Irritable aggression. Some dogs tend to react aggressively if someone tries to touch them or come close while they are resting. These dogs can become aggressive whenever the owner tries to brush them, medicate them or even simply tries to pet them. On a scale from 1 to 7 where 1 is 'never aggressive when disturbed/restrained' and 7 is 'very aggressive when disturbed/restrained', how would you define your dog's behaviour?

Never aggressive when disturbed/restrained ←————→ Very aggressive when disturbed/restrained

Without drug							
With drug							
	1	2	3	4	5	6	7

Q11. Tendency to avoid people or situations. Some dogs have a marked tendency to avoid people or situations that are unknown or unfamiliar, for example they tend to leave the room when unknown guests arrive or when people scream or there are loud noises. On a scale from 1 to 7 where 1 is 'no tendency to hide or avoid people or situations' and 7 is 'High tendency to hide or avoid people or situations', how would you rate your dog's behaviour?

No tendency to hide/avoid ←————→ High tendency to avoid

Without drug							
With drug							
	1	2	3	4	5	6	7

Q12. Mounting behaviour. Some dogs can show a tendency to mount people (children and adults) or other dogs, often of the same sex. On a scale from 1 to 7 where 1 is 'no tendency to mount' and 7 is 'high tendency to mount' how would you rate your dog's behaviour?

No tendency to mount ←————→ High tendency to mount

Without drug							
With drug							
	1	2	3	4	5	6	7

Appendix A

Did you identify other behaviours that your dog changed or started to show after the initiation of the drug?

(please provide details).....

THANK YOU FOR YOUR ASSISTANCE!

If you are willing to be contacted further about this research please provide your name and contact details below (e-mail or residential address)

.....

QUESTIONNAIRE ITALY

QUESTIONARIO

Questo questionario fa parte di un progetto di ricerca internazionale e la ringraziamo molto per aver accettato di compilarlo. Le informazioni in esso contenute resteranno confidenziali.

Lo scopo del questionario è raccogliere dei dati sui possibili effetti della terapia che sta somministrando al suo cane. Questi effetti potrebbero essere positivi/favorevoli oppure negativi/problematici per il suo cane e per la sua gestione. Lo studio riguarda il comportamento del cane durante la terapia. Le sue risposte verranno raggruppate con quelle di altri proprietari e non potranno venire direttamente attribuite a lei, garantendole in questo modo l'anonimato.

Data.....

NomeTel.....e-mail.....

Nome del cane.....Razza/Tipo.....Età.....M ☐ F ☐ Sterilizzata/Castrato ☐

Malattia/problema per il quale il cane assume il/i farmaci

Farmaco Dose* (vedere la nota sotto).....

*(Per favore, compili le informazioni sul farmaco includendo le istruzioni che ha ricevuto dal suo veterinario: dosaggio, intervallo tra le somministrazioni, cambiamenti di dosaggio nei giorni o settimane seguenti etc. La dose può essere in milligrammi o in compresse, specificando però la dose in ogni compressa. Per esempio se date mezza compressa dovrete specificare la dose in milligrammi contenuta in una compressa; es. mezza compressa da 5 mg per 2 volte al giorno)

Altri farmaci assunti nello stesso periodo (qualsiasi terapia, che sia o meno connessa con la malattia/problema che avete citato sopra, per esempio trattamenti antiparassitari o insulina se il vostro cane è diabetico)?

NO ☐ SI ☐

Farmaco Dose

Farmaco Dose

NEL RISPONDERE ALLE DOMANDE CHE SEGUONO, LA PREGHIAMO DI PENSARE, DAPPRIMA, AL COMPORTAMENTO DEL SUO CANE PRIMA DI INIZIARE LA TERAPIA E DI METTERE UNA X DOVE RITIENE OPPORTUNO NELLA STRISCIA BIANCA IN ALTO. POI DOVREBBE PENSARE AL COMPORTAMENTO DEL SUO CANE MENTRE STA PRENDENDO IL FARMACO E METTERE UNA X DOVE RITIENE PIU' APPROPRIATO, NELLA STRISCIA GRIGIA IN BASSO.

Q1. Comportamento giocoso. Alcuni cani sono molto motivati a giocare con le persone, con altri cani o con i loro giocattoli, per esempio hanno sempre voglia di inseguire una pallina, al parco corrono e giocano volentieri con gli altri cani e passano molto tempo a mordicchiare e portare in giro i loro giochi, mentre altri sono disinteressati o quasi indifferenti se vengono invitati a giocare dal padrone o dagli altri cani e non sono interessati ai giocattoli. In una scala da 1 a 7 dove 1 è 'per nulla giocoso' e 7 è 'molto giocoso' come classificherebbe il comportamento del suo cane?

Per nulla giocoso ←————→ *Molto giocoso*

Senza farmaco							
Con farmaco							
	1	2	3	4	5	6	7

Q2. Nervosismo/agitazione. Ci sono cani che si eccitano facilmente, ansimano, saltano addosso, abbaiano alle persone e agli altri cani e non riescono a stare tranquilli nella maggior parte delle situazioni, sia in casa che durante le passeggiate. Altri invece si agitano molto raramente e sono tranquilli anche in situazioni nuove o in presenza di persone e cani sconosciuti. Pensando al temperamento del suo cane, come lo definirebbe dal punto di vista del nervosismo/agitazione in una scala da 1 a 7 dove 1 è 'molto nervoso e agitato' e 7 è 'molto calmo'?

Molto nervoso e agitato ←————→ *Molto calmo*

Senza farmaco							
Con farmaco							
	1	2	3	4	5	6	7

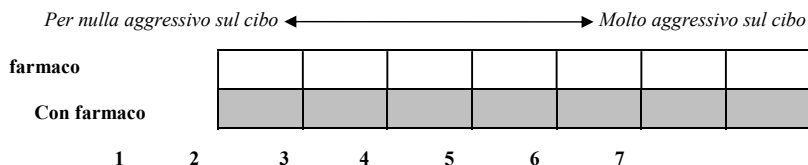
Q3. Insicurezza e paura. Pensando a come reagisce il suo cane in generale, per esempio, in presenza di persone sconosciute o di stimoli nuovi e sconosciuti (rumori, voci alte, posti che non conosce, animali che non conosce o bambini...), in una scala da 1 a 7 dove 1 è 'molto pauroso e insicuro' e 7 è 'molto sicuro di sé', come classificherebbe il suo cane?

Pauroso/insicuro ←————→ *molto sicuro di sé*

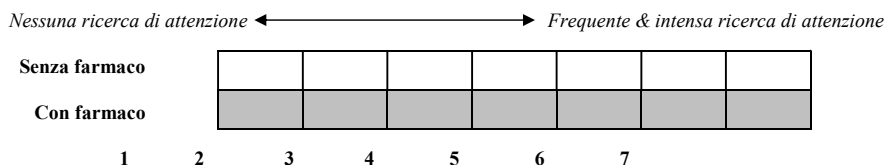
Senza farmaco							
Con farmaco							
	1	2	3	4	5	6	7

Appendix A

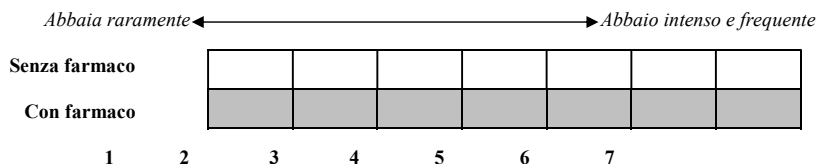
Q4. Aggressività in presenza di cibo. Alcuni cani ringhiano o addirittura tentano di pizzicare o mordere se qualcuno si avvicina alla loro ciotola del cibo, mentre altri sono completamente indifferenti e non hanno alcuna reazione neppure se la ciotola viene toccata o spostata. Pensando al comportamento del suo cane quando c'è del cibo, in una scala da 1 a 7 dove 1 è 'per niente aggressivo' e 7 è 'molto aggressivo' in presenza di cibo, come classificherebbe il suo cane?



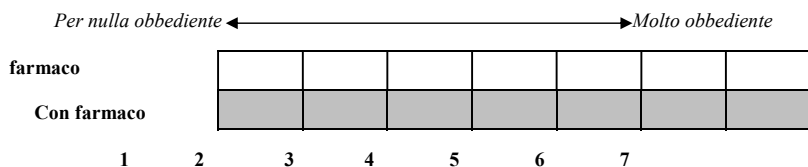
Q5. Comportamenti di ricerca di attenzione. Alcuni cani tendono a essere molto insistenti e cercare il contatto fisico con i proprietari saltando addosso, pizzicando, grattando con la zampa, uggiolando o abbaiano: in una scala da 1 a 7 dove 1 è 'nessun comportamento di ricerca di attenzione' e 7 è 'frequenti e intensi comportamenti di ricerca di attenzione', come classificherebbe il suo cane?



Q6. Abbaia. Alcuni cani abbaiano continuamente, giorno e notte, altri abbaiano solo in occasioni particolari. In una scala da 1 a 7 dove 1 è 'abbaia raramente' e 7 è 'abbaio intenso e frequente', come classificherebbe il comportamento del suo cane?



Q7. Obbedienza. Alcuni cani sono molto obbedienti, per esempio vengono prontamente se chiamati e vanno a cuccia quando viene loro chiesto, Mentre altri sono meno facilmente controllabili. In una scala da 1 a 7 dove 1 è 'per nulla obbediente' e 7 è 'molto obbediente', come classificherebbe il comportamento del suo cane?



Appendix A

Q8. Comportamento di guardia. Alcuni cani sono molto predisposti a fare la guardia e tendono a minacciare le persone abbaiano e ringhiando, altri sono amichevoli con tutti e non mostrano alcuna tendenza a fare la guardia. In una scala da 1 a 7 dove 1 è 'nessun comportamento di guardia' e 7 è 'intenso & frequente comportamento di guardia', come definirebbe il comportamento del suo cane?

Nessun comportamento di guardia ← → Comportamento di guardia intenso/frequente

Senza farmaco							
Con farmaco							
	1	2	3	4	5	6	7

Q9. Reazioni improvvise, di soprassalto. Alcuni cani tendono a trasalire molto facilmente, per esempio quando sentono un rumore o vengono toccati inaspettatamente. In questi casi possono reagire scappando, innervosendosi, ringhiando o magari con un pizzico o un morso improvviso. In una scala da 1 a 7 dove 1 è 'si allarma/trasale raramente' quando viene sorpreso e 7 è 'si allarma/trasale eccessivamente e frequentemente', come definirebbe il comportamento del suo cane in presenza di cose che non conosce?

Si allarma/trasale raramente ← → si allarma/trasale eccessivamente/frequentemente

Senza farmaco							
Con farmaco							
	1	2	3	4	5	6	7

Q10. Aggressività da irritazione. Alcuni cani tendono a reagire con un ringhio o magari tentando di pizzicare o mordere se qualcuno tenta di toccarli o si avvicina mentre stanno riposando. Questi cani possono diventare aggressivi quando il padrone vuole spazzolarli, medicarli o anche semplicemente accarezzarli. In una scala da 1 a 7 dove 1 è 'mai aggressivo quando disturbato/trattenuto/manipolato' e 7 è 'molto aggressivo quando disturbato/trattenuto/manipolato', come valuterebbe l'irritabilità del suo cane?

Mai aggressivo se disturbato ← → Molto aggressivo se disturbato

Senza farmaco							
Con farmaco							
	1	2	3	4	5	6	7

Q11. Tendenza a evitare le persone o certe situazioni. Alcuni cani hanno una marcata tendenza a evitare persone o situazioni sconosciute o poco familiari, per esempio tendono a lasciare la stanza quando arrivano ospiti sconosciuti o quando le persone gridano o ci sono rumori forti. In una scala da 1 a 7 dove 1 è 'nessuna tendenza a nascondersi o evitare persone o situazioni' e 7 è 'Spiccata tendenza a nascondersi o evitare persone o situazioni', Come classificherebbe il comportamento del suo cane?

Nessuna tendenza a nascondersi/evitare ← → Spiccata tendenza a nascondersi/evitare

Senza farmaco							
Con farmaco							
	1	2	3	4	5	6	7

Q12. Comportamento di monta. Alcuni cani mostrano una tendenza a montare le persone (Bambini e adulti) o altri cani, spesso dello stesso sesso. In una scala da 1 a 7 dove 1 è 'nessuna tendenza a montare' e 7 è 'spiccata tendenza a montare' come classificherebbe il comportamento del suo cane?

Nessuna tendenza a montare ← → Spiccata tendenza a montare

Senza farmaco							
Con farmaco							
	1	2	3	4	5	6	7

Appendix A

Ha identificato altri comportamenti che il suo cane ha modificato o ha iniziato a mostrare dopo l'inizio della terapia ?
(Per favore li
descriva).....

.....

GRAZIE PER IL SUO AIUTO!

Se ha piacere di essere contattato ancora riguardo a questa ricerca per favore scriva qui sotto dove vuole essere contattato (e-mail/ indirizzo/ numero di telefono)

.....

Appendix B

OWNERS' DISCLAIMER (ORIGINAL ITALIAN VERSION)

Appendix B

LIBERATORIA.

Test Comportamentale svolto il

Test 1: giorno _____ alle ore _____

Test 2: giorno _____ alle ore _____

Io sottoscritto/a _____ dichiaro di avere ricevuto tutte le informazioni riguardo al test al quale viene sottoposto oggi il mio cane di nome _____ razza _____ età _____ sesso _____ Sterilizzato SI ☐ NO ☐ e di avere accettato di partecipare liberamente e senza compensi in danaro.

Dichiaro inoltre di essere stato informato della possibilità di interrompere il test in qualsiasi momento, se lo ritenessi opportuno.

Acconsento a che le registrazioni del test vengano impiegate a scopo di analisi e possano essere mostrate in seminari o convegni scientifici, avendo ricevuto la rassicurazione che tutte le informazioni riservate riguardanti la mia identificazione personale rimarranno riservate.

In Fede



Dott. Lorella Notari

Medico Veterinario Comportamentalista

Dip. ECVBM-CA (Diploma Europeo in Medicina Comportamentale Veterinaria- Animali da Compagnia)

Via Donatell0, 6 21100 Varese Tel. +39 0332 286719 Cell. +39 335 5739223 lorellanotari@lorellanotari.it

OWNERS' DISCLAIMER (TRANSLATION INTO ENGLISH)

Appendix B

CONSENT.

Consent for behavioural tests on

Test 1 : _ date _____ time _____

Test 2 : _ date _____ time _____

My name _____ I declare I received the necessary information
about the behavioural tests that will be undertaken by my dog name _____ breed/type
_____ Age _____ Gender _____ Spayed/castrated YES ☐ NO ☐

and I accept to participate under my own will and without any payment .

I declare I was informed that I can stop the test in any moment .

I agree that the videorecordings of the tests will be used for behaviour analysis and that these recordings might
be shown in scientific seminars and meetings. I have been informed that alla my personal details will remain
confidential .

Signature



Dott. Lorella Notari

Medico Veterinario Comportamentista

Dip. ECVBM-CA (Diploma Europeo in Medicina Comportamentale Veterinaria- Animali da Compagnia)

Via Donatello, 6 21100 Varese Tel. +39 0332 286719 Cell. +39 335 5739223 lorellanotari@lorellanotari.it

Appendix C

PILOT STUDIES

CORTICOSTEROIDS AND DOG BEHAVIOUR: PILOT STUDIES

Before reaching the final version of our behavioural tests we made a few pilot studies in order to evaluate both the feasibility of dog sample recruiting and the reliability of the test design. Our goal was also to implement the test procedures.

The owners' questionnaire about possible effects of corticosteroids on dog behaviour (See Chapter 2 and 3) showed that dog in treatment with corticosteroid drugs tended to startle more easily, to be more nervous and more avoidant. The first pilot tests were therefore designed to experimentally verify these results.

The first pilots were designed with the goal of detecting dogs' reactions to acoustic stimuli. Our first attempts involved the use of a **Prepulse Inhibition (PPI)** test. PPI is a neurological phenomenon in which a weaker prestimulus (**prepulse**) inhibits the reaction to a subsequent strong startling stimulus (**pulse**). As acoustic stimuli we used the same prepulse and pulse stimuli used for horses in a previous study (¹). The rationale of this test was that dogs with more negative internal states might increase their startle reactions in response to the pulse compared with when they were not on corticosteroid therapy and compared with control dogs.

A simple apparatus was built, in order to be able to administer the stimuli (see figures below).

¹Van Der Laan, J. E. and Mills, D. S. (2011). Sensorimotor gating in equine stereotypy: prepulse inhibition of the acoustic startle reflex. Proceedings of the 7th International Equitation Science Conference. Academy Bartels - Hooge Mierde - The Netherlands



(a)



(b)



(c)

(a) Dog's view. The owner will sit on the chair. (b) Loudspeaker positions. The holes are at different levels for different dog's sizes. (c) The dog is held by his/her owner through the screen and the owner tries to keep the dog's head in the correct position by showing a titbit.

Figure C.1: PPI apparatus.

A few healthy dogs were used to test the apparatus but this test was discarded because most dogs appeared distressed by the apparatus itself and the owner's attempts to hold them in the right position for receiving the sound stimuli in most cases seemed more salient than the administered stimuli itself. Because we expected that the sample would not be easy to recruit and we had to be able to successfully test dogs on corticosteroid without losing too many of them due to overly complicated procedures we decided to change the test design.

A first version of the test that we finally used was introduced. To test the tendency of dogs to avoid a potentially mild aversive stimulus we designed a first version of the

test as in Figure C.2.

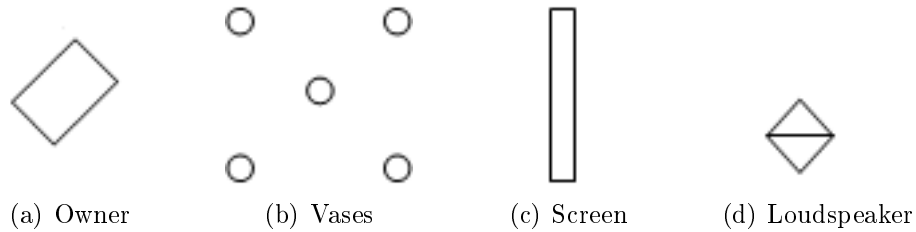


Figure C.2: Test area with vases.

The test area was firstly set in a room of about 20 square meters, with five vase arranged on the floor as in Figure C.1. A sound started from a loudspeaker positioned behind a screen.

Practical problems arose in the first test trials. The first problem was to find a sound with the intrinsic intention of being mildly aversive to all dogs. The kind and intensity of sounds were changed many times before reaching the decision to introduce the growls that we used in the final version of the tests.

The vase position was changed mainly in anticipation of having to make tests in smaller environments such as veterinary practices. A single line of vases in front of the screen that hid the loudspeakers took up less space and enabled us to put pieces of food on both sides of the line at the same time.

The recruitment of dogs was a very challenging part of the study and different methods were considered including e-mails, social network advertising and the involvement of training centers. The final decision to limit the recruitment of sample dogs to dogs that had just received a prescription for corticosteroid drugs led us to the decision to contact veterinary clinics in the area.

Appendix D

DOG BEHAVIOUR FORM

**SCHEDA INFORMATIVA PER IL
CANE
(ORIGINAL ITALIAN VERSION)**

Data consultazione...

Caso n.

INFORMAZIONI GENERALI

Nome.....Indirizzo.....Città.....CAP.

Tel. Casa.....

Cellulare.....

e-mail.....

Veterinario curante/clinica.....Tel.....

Nome del cane... ..

Razza/tipo.....età.....sesso....

È sterilizzato/a ?.....

Se sì, quando è stato sterilizzato Sì ☐ No ☐

LA STORIA

Età di adozione?

da dove proviene?

Sono suoi primi proprietari o è stato precedentemente in un'altra famiglia o in canile?

.....

Adozione dal canile: notizie precedenti

LA DIETA

Che tipo di alimentazione segue il suo cane ? Casalinga ☐ Industriale ☐ Mista ☐
Quante volte al giorno?
Quando? (ora e quando rispetto al pasto dei proprietari)
Da qualche integratore? Se sì, cosa?
Mangia con entusiasmo o è schizzinoso?
bocconcino-premio? Se sì, cosa? Decrivere

ESERCIZIO, GIOCHI E INTERAZIONI CON GLI ALTRI CANI

Tipo di movimento (es. solo in giardino, segue il proprietario che fa jogging, in campo di addestramento, agility o altri sport). Descrivere.....
Quante ore/minuti di movimento al giorno?.....
da solo o con altri cani?.....
al guinzaglio o libero?...
Gli piace uscire in passeggiata? Sì ☐ No ☐ Dipende ☐ (descrivere)
.....
Ci sono interazioni/giochi con altri cani? Sì ☐ No ☐ Dipende ☐ (descrivere)
.....
Qual è il giocattolo preferito?
Qual è il suo gioco preferito con le persone?,
Dove tiene i giocattoli del cane?.....
Il cane può accedere liberamente ai giocattoli? Sì ☐ No ☐ Dipende ☐ (descrivere)
.....

SISTEMAZIONE IN CASA

Dove dorme la notte? Fuori casa ☐ In casa ma non in camera ☐ in camera ☐ altro

Il cane ha una sua cuccia o brandina e lo usa per dormire di notte e riposare ? Si ☐ No ☐

Se no, descrizione di dove dorme il cane di notte e dove riposa di giorno

.....

Dove sta il cane quando sono fuori casa? In giardino ☐ In casa ☐ Altro.....

È lasciato solo regolarmente? Se si, per quanto tempo?.....

Ci sono problemi quando lo lasciano solo? Che cosa succede? (Descrizione).

Lasciano giocattoli o qualche altra distrazione? Si ☐ No ☐ Cosa?

Ha accesso al giardino? Si ☐ No ☐

Quando ci sono, il cane tende a seguire per la casa? Si ☐ No ☐

LA STORIA DELLA SUA EDUCAZIONE/ADDESTRAMENTO

Ha seguito qualche corso di educazione/addestramento? Si ☐ No ☐

Che età aveva allora il cane?

Per quanto tempo?

Era un corso singolo o c'erano altri cani? Singolo ☐ Collettivo ☐

Ci sono stati problemi? Si ☐ No ☐ Quali?

Come ha insegnato al cane a sporcare fuori casa? (Descrivere)

Il cane tira al guinzaglio? Si ☐ No ☐ Dipende ☐ (descrivere)

Torna al richiamo? Si ☐ No ☐ Dipende ☐ (descrivere)

Lascia oggetti che tiene in bocca quando richiesto? Si ☐ No ☐ Dipende ☐ (descrivere)

Quali comandi conosce il cane /cosa sono in grado di chiedere al cane (es. vieni, seduto, resta...)?

MEMBRI DELLA FAMIGLIA

Quante persone vivono in casa? Ci sono bambini? Se si, quanti anni hanno? Descrizione dei componente della famiglia (età, sesso, grado di parentela)

Tutti i membri della famiglia interagiscono con il cane? Descrizione (per esempio chi porta fuori il cane, chi gli da il cibo, chi lo spazzola etc.)

Altri animali (Descrizione del tipo, età, sesso) ?

Quando sono stati adottati questi animali? Età di adozione/quando sono arrivati in famiglia

OSPITI

Quando arrivano ospiti/sconosciuti come si comporta? Amichevole ☐ ha paura ☐ aggressivo ☐
altro ☐ (descrivere)

Si comporta in modo diverso a seconda del tipo di ospite (per esempio, maschi, femmine, bambini).
Descrivere

SPAZZOLATURE E MANIPOLAZIONI

Come si comporta quando viene spazzolato, per pulire le orecchie, per lavarlo?

COMPORTAMENTO DAL VETERINARIO

Come si comporta dal veterinario? (descrivere, per es. molto pauroso, aggressivo, deve sempre mettere la museruola)

DESCRIZIONE DEL PROPRIETARIO DELLA PERSONALITA' DEL CANE

Aggressivo in molte/nella maggior parte delle situazioni? Si ☐ No ☐.

Spesso nervoso o spaventato (per rumori, cose nuove, estranei)?	Si	<input type="checkbox"/>	No	<input type="checkbox"/>
Prevalentemente vivace ed entusiasta?	Si	<input type="checkbox"/>	No	<input type="checkbox"/>
Socievole?	Si	<input type="checkbox"/>	No	<input type="checkbox"/>
Sicuro di sé?	Si	<input type="checkbox"/>	No	<input type="checkbox"/>

ANAMNESI CLINICA E VISITA CLINICA

Il cane ha in questo momento problemi di salute ? Si ☐ No ☐ Quali?.....

Ha avuto problemi di salute in passato? Si ☐ No ☐ Quali problemi ha avuto? Elenco breve

.....

.....

.....

Quali farmaci ha assunto per questi problemi?

Nome del farmaco 1.....Dosaggio.....

per quanto tempo?

Meno di una settimana ☐

Più di una settimana ☐

Terapia ad vitam o per periodi molto lunghi ☐

Nome del farmaco 2.....Dosaggio.....

per quanto tempo?

Meno di una settimana ☐

Più di una settimana ☐

Terapia ad vitam o per periodi molto lunghi ☐

Visita clinica:

Visita non svolta ☐

Impossibile da visitare senza sedazione ☐

Visitato con museruola ☐

Stato di Nutrizione (BCS) 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

Condizioni del mantello Ottime ☐ Buone ☐ Scarse ☐ (descrivere) Pessime ☐
(descrivere)

Battito e respiro Nella norma ☐ Alterazioni ☐ (descrivere).....

Addome Normale/palpabile ☐ non palpabile ☐ alterazioni (descrizione)

Linfonodi Normali ☐ Alterazioni (descrizione).....

Temperatura rettale (°)

Orecchie – aspetto della pinna e del condotto uditivo esterno

Orecchie temperatura DX(°).... SX(°)

IL PROBLEMA PRINCIPALE

Descrizione del comportamento

Insorgenza del problema

Il comportamento è rimasto lo stesso o è peggiorato nel tempo?

Descrizione dell'ultimo episodio/incidente

Appendix D

Eventi precedenti e seguenti

Dove è accaduto, quando è accaduto (giorno e ora) e chi era presente

Contesti a rischio in casi di aggressività (es. passeggiata, arrivo di ospiti, presenza
Di bambini, presenza di altri cani) . Elencare

Il problema si presenta soprattutto se...

Cronologia degli episodi/ incidenti avvenuti in passato. Descrizione

Stato dell'animale (es. Calore, gravidanza, malattie)

ALTRI PROBLEMI/COME SI COMPORTA:

Con i bambini

Con gli estranei

Con i membri della famiglia

Quando viene spazzolato o lavato

Quando gli danno mangiare

Con i gatti

Quando ci sono rumori forti

Quando incontra altri cani

CI SONO ALTRI PROBLEMI NON ANCORA DESCRITTI/RIPORTATI?

PRESCRIZIONI

ISTRUZIONI CONSEGNATE AL TERMINE DELLA VISITA



EtoStudio
Dott. Lorella Notari

Medico Veterinario Comportamentista

Dip. ECAWBM-CA (Diploma Europeo in Medicina Comportamentale Veterinaria- Animali da Compagnia)

Via Donatello, 6 21100 Varese Tel. +39 0332 286719 Cell. +39 335 5739223

lorellanotari@etostudio.it

DOG BEHAVIOUR FORM

(ENGLISH TRANSLATION)

Appendix D

Date of consultation...

Case n.

GENERAL INFORMATION

Name.....Address.....City.....CAP.

Tel. Home.....

Mobile.....

e-mail.....

Veterinary surgeon/practice.....Tel.....

Dog name... ..

Breed/type.....age.....gender....

Is the dog spayed/castrated ? Si ☐ No ☐

I yes when?

DOG BACKGROUND

Adoption age?

Where does the dog come from?

Are they the first owners or was the dog rehomed from another family or a rescue kennel?

.....

In the case of adoption from a rescue kennel: information about previous dog's life

THE DIET

What kind of diet is the dog taking ? Home made ☐ Industrial food ☐ A mix ☐
How many times per day is the dog fed?
When? (Time of the day)
Any supplement? If yes, what?
Is the food eaten with enthusiasm?
Any titbit? If yes, what? Describe

EXERCISE, PLAY AND INTERACTION WITH OTHER DOGS

Type of exercise (for example only in the owner's garden, jogging with the owner, walk in town or country, agility or other sports). Description.....
How many hours/minutes of exercise per day?.....
Are there other dogs?.....
On leash or also free from the leash?... ..
Does he/she like to go out? Yes ☐ No ☐ Depends ☐ (describe)
Are there interactions/play with other dogs? Yes ☐ No ☐ Depends ☐ (description)
What is the dog's favourite toy?
What is the dog's favourite game with people?,
Where are the dog's toy kept?.....
Has the dog free access to his/her toys? Yes ☐ No ☐ Depends ☐
(description)

MANAGEMENT AT HOME

Where does the dog sleep during the night? Outdoor ☐ In the house but not in the bedroom ☐ in the bedroom ☐ other

Does the dog have its own bed and use it for sleeping or resting ? Yes ☐ No ☐ If no, description of where the dog sleeps or rest at night or during the day

Where is the dog when the owners are away from home? outdoor ☐ indoor ☐ Other.....

Is the dog left alone regularly? If yes, how long?.....

Are there problems when the dog is left alone? What happen? (Description).

Do they leave toys or other distractions? Yes ☐ No ☐

What?

Has the dog free access to the garden? Yes ☐ No ☐

When owners are at home, does the dog follow them all the time? Yes ☐ No ☐

HISTORY OF DOG TRAINING

Did you follow training courses with your dog Yes ☐ No ☐

What age was the dog?

How long did you follow the course?

Were there other dogs on the course? Private/single course ☐ Collective ☐

Problems during the training? Yes ☐ No ☐ What problems?

How was the dog housetrained? (description)

Does the dog pull on the lead? Yes ☐ No ☐ Depends ☐ (description)

Appendix D

Does the dog come when called? Yes ☐ No ☐ Depends ☐ (description)

Does the dog leave object when asked? Yes ☐ No ☐ Depends ☐ (description)

What words/commands can the dog understand /what are the owners are able to ask the dog (for example come, sit, stay)?

FAMILY MEMBERS

How many people live in the house? Children? If yes, what age are they? Description of family members (age, gender, degree of relationship)

Do all the family members interact with the dog? Description of type of interactions (for example who walks the dog, who feeds the dog, who grooms the dog)

Other pets (Species, type, age, gender) ?

Appendix D

When were these other pets introduced? Age of adoption/when they arrived in the family

GUESTS

How does the dog behave when guests arrive? Friendly ☐ fearful ☐ aggressive ☐ other ☐
(description)

Does the dog behave differently with different guests (for example male, female, children).
Description

GROOMING AND HANDLING

How does the dog behave when being groomed, ear cleaning, washing?

BEHAVIOUR DURING VETERINARY VISITS

How the dog behave with vets? (for example is very fearful, aggressive, there is a need to wear a muzzle..)

HOW WOULD YOU DESCRIBE YOUR DOG PERSONALITY?

Aggressive in most situations? Yes ☐ No ☐.

Do you think that your dog is often or always nervous or fearful in the presence of unknown situations or stimuli (sounds, new stimuli, unknown people or dogs)?

Yes ☐ No ☐

Do you consider your dog largely enthusiastic and excited?

Yes ☐ No ☐.

Do you think that your dog is sociable in general?

Yes ☐ No ☐

Do you consider your dog confident?

Yes ☐ No ☐.

CLINICAL HISTORY AND CLINICAL EXAMINATION

Does the dog have health problems at the moment ? Yes ☐ No ☐ What?.....

Has the dog had any health problems in the past? Yes ☐ No ☐ What kind of problems? Brief list

.....

.....

.....

What drugs did your dog take for these problems? List the most recent drug used

Name of drug 1.....Dose.....

How long did the dog take the drug?

Less than a week ☐

More than a week ☐

Therapy ad vitam or for very long periods ☐

Name of drug 2.....Dose.....

How long did the dog take the drug?

Less than a week ☐

More than a week ☐

Therapy ad vitam or for very long periods ☐

Clinical examination:

No clinical examination ☐

Impossible to examine ☐

Need a muzzle to be visited ☐

Nutritional status (BCS) 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

Coat condition very good ☐ good ☐ poor ☐ (description) very poor ☐ (description)

Hearth and respiratory rates Normal ☐ Alterations ☐
(descriptions).....

Abdomen Normal/palpable ☐ non palpable ☐ Alterations (description)

Lymphnodes Normal ☐ Alterations (description).....

Rectal temperature (°)

Ears – pinna and external duct aspect

Ear temperature DX(°).... SX(°)

MAIN BEHAVIOUR PROBLEM

Description of the behaviour

When did it start

As the behaviour remained the same or deteriorated over time?

Description of the last episode

Appendix D

What happened before and after (including what people/owner did before and after)

Where did it happen, when (day and time) and who was present

Risky contexts when aggression is involved (for example during walks, when guests arrive, in the presence of children, in the presence of other dogs...) . List these contexts

The problem arises especially if...

Chronology of past episodes and descriptions

Physiological condition (for example health, pregnancy, diseases)

OTHER BEHAVIOURS. HOW THE DOG BEHAVES:

with children

with strangers

with family members

when groomed or washed

when it is fed

with cats

When there are loud noises

Appendix D

With other dogs

ARE THERE OTHER PROBLEMS THAT HAVE NOT BEEN MENTIONED?

PRESCRIPTIONS

INSTRUCTIONS GIVEN AT THE END OF THE CONSULTATION



Dott. Lorella Notari
Medico Veterinario Comportamentista
Dip. ECAWBM-CA (Diploma Europeo in Medicina Comportamentale Veterinaria- Animali da
Compagnia)
Via Donatello, 6 21100 Varese Tel. +39 0332 286719 Cell. +39 335 5739223
lorellanotari@etostudio.it

Appendix E

DESCRIPTIVE STATISTICS

CHAPTER 5. TABLES

- AGE AND GENDER DISTRIBUTIONS IN THE DIFFERENT MEDICAL CONDITIONS

Dermatological Conditions

Age	Frequency n. (%)
1-2 years	36 (40,4)
3-4 years	32 (36,0)
5-6 years	12 (13,5)
7-8 years	8 (9,0)
9-10 years	1 (1,1)

Table E.1: Age distribution of dogs with a history of dermatological condition.

Gender and Reproductive State	Frequency n. (%)
entire male	40 (44,9)
entire female	15 (16,9)
castrated male	16 (18,0)
spayed female	18 (20,2)

Table E.2: Gender and reproductive state distribution of dogs with a history of dermatological conditions.

Orthopaedic Condition

Age	Frequency n. (%)
1-2 years	4 (14,8)
3-4 years	10 (37,0)
5-6 years	2 (7,4)
7-8 years	9 (33,3)
9-10 years	2 (7,4)

Table E.3: Age distribution of dogs with a history of orthopaedic condition.

Gender and Reproductive State	Frequency n. (%)
entire male	14 (51,9)
entire female	3 (11,1)
castrated male	2 (7,4)
spayed female	8 (29,6)

Table E.4: Gender and reproductive state distribution of dogs with a history of orthopaedic conditions.

Gastroenterical Conditions

Age	Frequency n. (%)
1-2 years	51 (64,6)
3-4 years	17 (21,5)
5-6 years	9 (11,4)
7-8 years	2 (2,5)

Table E.5: Age distribution of dogs with a history of gastrointestinal conditions.

Gender and Reproductive State	Frequency n. (%)
entire male	38 (48.1)
entire female	11 (13.9)
castrated male	7 (8.9)
spayed female	23 (29.1)

Table E.6: Gender and reproductive state distribution of dogs with a history of gastrointestinal conditions.

- **AGE, GENDER AND AFFECTIVE STATES**

Positive Affective States

Age	Positive affect Frequency n. (%)
1-2 years	40 (78.4)
3-4 years	8 (15.7)
5-6 years	3 (5.9)
7-8 years	0
9-10 years	0

Table E.7: Age distribution of dogs in positive affective states.

Gender and Reproductive State	Positive affect Frequency n. (%)
entire male	22 (43.1)
entire female	15 (29.4)
castrated male	7 (13.7)
spayed female	7 (13.7)

Table E.8: Gender of dogs in positive affective states.

Negative Affective States

Age	Negative affect Frequency n. (%)
1-2 years	155 (53.1)
3-4 years	79 (27.1)
5-6 years	29 (9.9)
7-8 years	26 (8.9)
9-10 years	3 (1.0)

Table E.9: Age distribution of dogs in negative affective states.

Gender	Negative affect Frequency n. (%)
entire male	155 (53.1)
entire female	41 (14.0)
castrated male	28 (9.6)
spayed female	68 (23.3)

Table E.10: Gender of dogs in negative affective states.

• AGE, GENDER AND AGGRESSION TOWARDS PEOPLE

Age	Agg. Frequency n. (%)
1-2 years	69 (46,6)
3-4 years	45 (30,4)
5-6 years	21 (14,2)
7-8 years	12 (8,1)
9-10 years	1 (0,7)

Aggr.= aggression towards people.

Table E.11: Age distributions of dogs presented for aggression towards people.

Gender	Agg. Frequency n. (%)
entire male	95 (64,2)
entire female	14 (9,5)
castrated male	19 (12,8)
spayed female	20 (13,5)

Aggr.= aggression towards people.

Table E.12: Gender of dogs presented for aggression towards people.

• **AGE, GENDER AND HYPERACTIVITY**

Age	Hyperactivity Frequency n. (%)
1-2 years	73 (82,0)
3-4 years	10 (11,2)
5-6 years	3 (3,4)
7-8 years	3 (3,4)

Table E.13: Age distributions of dogs presented for hyperactivity.

Gender	Hyperactivity Frequency n. (%)
entire male	41 (46,1)
entire female	18 (20,2)
castrated male	9 (10,1)
spayed female	21 (23,6)

Table E.14: Gender of dogs presented for hyperactivity.

- **AGE, GENDER AND PRESCRIPTION OF PSYCHOACTIVE DRUGS**

Age	Psychoactive Frequency n. (%)
1-2 years	46 (43,0)
3-4 years	32 (29.9)
5-6 years	15 (14.0)
7-8 years	13 (12.1)
9-10 years	1 (0.9)

Psychoact = psychoactive drug prescriptions.

Table E.15: Age distribution of dogs that received a prescription for psychoactive drugs.

Gender	Psychoactive Frequency n. (%)
entire male	46 (43.0)
entire female	17 (15.9)
castrated male	19 (17.8)
spayed female	25 (23.4)

Psychoact = psychoactive drug prescriptions.

Table E.16: Gender of dogs that received a prescription for psychoactive drugs.

- **MEDICAL CONDITIONS AND AFFECTIVE STATES**

Medical condition	Positive affect Frequency n. (%)
Dermatological problems	7 (13.7)
Orthopedic problems	1 (2.0)
Gastrointestinal problems	17 (33.3)
Respiratory problems	9 (17.6)
Other medical problems	4 (7.8)

Table E.17: Medical conditions of dogs in positive affective states.

Medical Condition	Negative affect Frequency n. (%)
Dermatological problems	82 (28.1)
Orthopedic problems	27 (7.9)
Gastrointestinal problems	62 (21.2)
Respiratory problems	46 (15.8)
Other medical problems	30 (10.3)

Table E.18: Medical conditions of dogs in negative affective states.

• MEDICAL CONDITIONS AND AGGRESSION TOWARDS PEOPLE

Medical condition	Agg. Frequency n. (%)
Dermatological problems	43 (29.1)
Orthopedic problems	15 (10.1)
Gastrointestinal problems	22 (14.9)
Respiratory problems	26 (17.6)
Other medical problems	16 (10.8)
No history of medical problems	36 (24.3)

Aggr.= aggression towards people.

Table E.19: Medical conditions of dogs presented for aggression towards people.

• MEDICAL CONDITIONS AND HYPERACTIVITY

Medical condition	Hyperactivity Frequency n. (%)
Dermatological problems	16 (18.0)
Orthopedic problems	2 (2.2)
Gastrointestinal problems	27 (30.3)
Respiratory problems	18 (20.2)
Other medical problems	0
No history of medical problems	30 (33.7)

Table E.20: Medical conditions of dogs presented for hyperactivity.

- **MEDICAL CONDITIONS AND PRESCRIPTION OF PSYCHOACTIVE DRUGS**

Medical condition	Psychoactive Frequency n. (%)
Dermatological problems	36 (33.6)
Orthopedic problems	9 (8.4)
Gastrointestinal problems	22 (20.6)
Respiratory problems	17 (15.9)
Other medical problems	11 (10.3)
No history of medical problems	20 (18.7)

Table E.21: Medical conditions of dogs that received a prescription for psychoactive drugs.

Patient Factors	History of corticosteroid treatment		Pearson Chi Square X(1)	p value
	Positive n. (%)	Negative n. (%)		
Positive affective state	0 (0.0)	36 (17.2)	–	–
Negative affective state	55 (100.0)	173 (82.8)	10.970	< 0.001
Aggression towards people	31 (56.4)	81 (38.8)	5.527	0.014
Hyperactivity	4 (7.3)	55 (26.3)	9.099	< 0.001
Aggression towards other dogs	9 (16.4)	41 (19.6)	0.300	0.370
Separation problems	9 (16.4)	30 (14.4)	0.140	0.425
Phobias	13(23.6)	56 (26.8)	0.225	0.387
Excessive barking	5 (9.1)	27 (12.9)	0.599	0.303
History of severe bites	16 (29.1)	47 (22.5)	1.045	0.198
Psychoactive drug prescription	24 (43.6)	63 (30.1)	3.588	0.043

Table E.22: Relationship between history of treatment with corticosteroids and negative affective state, reported behaviour problems, occurrence of sever bites and the prescription of psychoactive drugs in all dogs that had a history of medical problems that required more than one week of therapy (n. 264).

Appendix F

BREED GROUPS

The Fédération Cynologique Internationale and breed groups.

The Fédération Cynologique Internationale (FCI) is the World Canine Organisation.

The FCI recognises **343 breeds**. Each of them is the “property” of a specific country. The “owner” countries of the breeds write the standard of these breeds (detailed description of the ideal type of the breed), in co-operation with the Standards and Scientific Commissions of the FCI. The translation, updating and publication of the standards are carried out by the FCI. These standards are the reference for the judges at shows held in the FCI member countries, but also for the breeders in their attempt to produce top-quality dogs.

Breed groups

FCI recognized breeds are divided into 10 groups. A brief description of these groups and a brief list of the most popular breeds for each group are listed below.

- **GROUP 1**

Sheepdogs and Cattledogs (except Swiss Cattledogs)

German Shepherd

Belgian Shepherd

Czechoslovakian Shepherd

Border Collie

Maremmano Abruzzese Shepherd

- **GROUP 2**

Pinscher and Schnauzer - Molosoid and Swiss Mountain and Cattledogs

Rottweiler

Boxer

Doberman

Cane Corso

British Bulldog

Great Dane

Schnauzer

- **GROUP 3**

Terrier

American Staffordshire Terrier

Bull Terrier

West Highland White Terrier

Yorkshire Terrier

Fox Terrier

- **GROUP 4**

Dachshunds

- **GROUP 5**

Spitz and primitive types

Siberian Husky

Akita Inu

Samoyed

Chow-Chow

Italian Spitz (Volpino Italiano)

- **GROUP 6**

Scent hounds and related breeds

Italian Scenthound

Beagle

Dalmatian

Basset Hound

- **GROUP 7**

Pointing Dogs

Pointer

Irish Setter

- **GROUP 8**

Retrievers – Flushing Dogs – Water Dogs

Labrador Retriever

Golden Retriever

Cocker Spaniel

Springer Spaniel

- **GROUP 9**

Companion and Toy Dogs

Chihuahua

Shih Tzu

Cavalier King Charles

Pekinese

Poodles

Bolognese

- **GROUP 10**

Sighthounds

Greyhound

Galgo

Italian Greyhound (Piccolo Levriero Italiano)

Appendix G

CHECK SHEET FOR VETERINARY PRACTITIONERS

DOG BEHAVIOUR CHECK LIST:

IMPORTANT THINGS TO CHECK WHEN A DOG IS BEING PRESCRIBED

CORTICOSTEROID DRUGS

*This check sheet is intended as an aid for practitioners, who may be unfamiliar with the behavioural side effects of corticosteroid drugs. The sheet focuses on the areas of behavioural change in the dog which have been found to be of significance and relevance to human safety. **Please use this to help control preventable risk.***

Glucocorticoid drugs can change the behaviour of normal dogs and those with pre-existing behaviour problems. You should ask your clients a few simple questions before they start the corticosteroid treatment and provide at least the concise advice outlined below to help to prevent possible problems as necessary. In extreme cases you may wish to reconsider the benefits of corticosteroid therapy versus the risks, before the animal has received specialist behavioural support.

Part 2 relates to a follow up session when potential changes in behaviour might have been noticed by owners during the treatment. This latter information might be very useful in the case of repeated courses of treatment in order to prevent the onset or worsening of management and behaviour problems in future.

Case n. Client name Tel/E-mail.....
Dog name.....Age..... Gender M ☐ F ☐ Neutered ☐ Breed/type
Drug prescribedDose..... Reason for prescription.....

PART 1. Prescription time. Start of the therapy (day/month/year) .././....

Question 1. Behaviour in the presence of food

Did your dog ever growl or behave aggressively towards human beings in the presence of food?

Yes ☐ No ☐

Advice

While your dog receives therapy with (name of the drug) he/she might be more motivated to defend food. Regular rituals for feeding your dog will prevent unexpected aggressive reaction for example when someone comes close to his/her bowl or when the dog

is approached while he/she is chewing something. Give food in a quiet area of your house, take away the bowl when the dog has finished and is far removed from his/her feeding place. Supervise young children so that they will not have any opportunity to come close to the dog when there is food or chewing objects around. If you answered 'Yes' to this question, the above suggestions are important all the time and not just during corticosteroid therapy: consider seeking professional behavioural advice to address this problem more fully.

Question 2. Fears

Is your dog fearful of people, sounds or particular stimuli?

Yes ☐ No ☐

Advice

While your dog receives therapy with(name of the drug) he/she might increase fear related reactions. If your dog is shy with people he/she might be more motivated to avoid physical contacts and this might include a higher tendency to withdraw when approached or even try to snap or bite. If you notice that your dog tends to withdraw when approached ask people not to touch him/her and be particularly careful with children. In the same way, if your dog is sensitive to sounds, try to limit exposure to unusually noisy environments during the therapy. If your dog is fearful of thunderstorms this might worsen during the therapy: keep the dog in a quiet and protected place and avoid contact with children and strangers in these situations. If your dog shows severe signs of fear consider seeking professional advice to address these problems more fully.

Question 3. Irritability

Does your dog growl when disturbed? For example when someone touches him/her while he/she is resting or playing?

Yes ☐ No ☐

Advice

While your dog receives therapy with(name of the drug) he/she might become more irritable.

This tendency might also be related to physical discomfort and it is important to prevent unpredictable physical contacts. Always ask your dog to come towards you rather than go to his/her bed and pet him/her. Instruct all your family members to do the same. It is also important that children avoid hugging or holding your dog while he/she is not well and receiving therapy with corticosteroid drugs. If your dog growled when touched in the past, prevent children and strangers from touching him/her. If your dog has growled in the past when being groomed put on a muzzle before grooming. If you answered yes to Question 3, consider seeking professional advice for a fuller resolution of this issue.

Question 4. Barking

Does your dog bark frequently and intensively?

Yes ☐ No ☐

Advice

While your dog is receiving therapy with(name of the drug) he/she might bark more.

To prevent this problem try to individualise the contexts in which the dog tends to bark more and the motivation for barking. If barking is mainly motivated by alarm or territorial defense try to keep your dog far from your garden fence and in general try to protect him/her from any triggering stimuli. This might prevent a worsening of this behaviour. If barking is mainly motivated by attention seeking try not to reinforce this behaviour with your attention (this includes shouting at the dog in an attempt to tell him/her off) and increase positive reinforcements when the dog is calm. Excessive barking can be a sign of distress and professional advice might be indicated in severe cases.

PART 2. Follow up. End of the therapy (day/month/year) ../../....

A few possible changes in behaviour during glucocorticoid therapies might be reported by owners

Some possible changes are listed below. Filling in this part of the checklist will be a useful tool for giving advice in case of repeated courses of therapy with corticosteroid drugs.

During the therapy the dog was:

- | | | | | | |
|------------------------|--------------------------|----------------------|--------------------------|------------------------------|--------------------------|
| • Less playful | <input type="checkbox"/> | More playful | <input type="checkbox"/> | Playful as usual | <input type="checkbox"/> |
| • More food protective | <input type="checkbox"/> | Less food protective | <input type="checkbox"/> | No change in food protection | <input type="checkbox"/> |
| • More fearful | <input type="checkbox"/> | Less fearful | <input type="checkbox"/> | No changes in fearfulness | <input type="checkbox"/> |
| • More irritable | <input type="checkbox"/> | Less irritable | <input type="checkbox"/> | No changes in irritability | <input type="checkbox"/> |
| • Barked more | <input type="checkbox"/> | Barked less | <input type="checkbox"/> | No changes in barking | <input type="checkbox"/> |

Appendix H

PUBLISHED PAPERS



ELSEVIER

RESEARCH

Possible behavioral effects of exogenous corticosteroids on dog behavior: a preliminary investigation

Lorella Notari, Daniel Mills

*Animal Behaviour, Cognition and Welfare Group, Department of Biological Sciences, University of Lincoln, Riseholme Park, United Kingdom.***KEYWORDS:**behavior;
corticosteroids;
dog;
prednisolone;
side effects

Abstract Glucocorticoids are widely used in veterinary medicine and their physical side effects are well-known; however, the effects on dog behavior linked to their role in the stress response and effects on mood have not been reported in previously published data. In this article, retrospective owner reports of the behavioral changes in dogs during corticosteroid therapy in a series of cases have been described so as to generate items for future use in a controlled structured questionnaire. The perceptions of behavioral changes in dogs during corticosteroid therapy were investigated through semi-structured open interviews of the owners of 31 dogs of different breeds, genders, and ages. All dogs had received corticosteroid therapies in the past 6 months. In all, 18 dogs had been administered methylprednisolone (dose range, 0.2-1 mg/kg), 8 were administered prednisolone (dose range, 0.2-1 mg/kg), and 5 were administered dexamethasone (dose range, 0.01-0.3). Methylprednisolone and prednisolone were used for dermatological conditions, and dexamethasone was used for orthopedic conditions. Owners were asked to describe their dog's behaviors both on and off corticosteroid therapy. Interviews were ceased when answers became repetitive with no new reported behavioral change (interview to redundancy). In all, 11 owners reported behavioral changes in their dogs; 9 dogs were reported to show more than one behavioral change. Six dogs reportedly showed nervousness and/or restlessness, 3 showed an increase in startle responses, 3 showed food guarding, 2 showed a decrease in their activity level, 3 showed an increase in avoidance responses, 4 showed irritable aggression, and 2 dogs increased barking. Semi-structured interviews can be useful preliminary tools for the identification of areas of future investigation, and the outcomes of the interviews reported in this article will be used in further quantitative research, to investigate more rigorously the possible relationship between these signs and corticosteroid use in dogs. © 2011 Elsevier Inc. All rights reserved.

Introduction

Corticosteroids are widely used in veterinary medicine for several conditions and they are among the most prescribed drugs for pet animals (McDonald and Langston, 1995, Sousa, 2009).

Address for reprint requests and correspondence: Lorella Notari, Animal Behaviour, Cognition and Welfare Group, Department of Biological Sciences, University of Lincoln, Riseholme Park LN2 2LG, UK; Tel: +441522895356.

E-mail: lorellanotari@lorellanotari.it

Corticosteroid drugs have both mineralocorticoid and glucocorticoid activities, but are mainly used for their glucocorticoid effects. It is well-known and also well reported that exogenous corticosteroids can lead to medical complications, either directly or indirectly, by causing, for example, immunosuppression or masking signs that might be important for the diagnosis or monitoring of a disease; however, they are also important components of stress responses. Evidence from other species suggests that effects on the brain are complex, involving multiple neurotransmitters and a range of cognitive processes and behavioral

responses (Wolkowitz, 1994). Although the physical side effects of corticosteroids have been widely described in veterinary medicine, reports of psychological or behavioral side effects are anecdotal (Dodman and Schuster, 1998) and, so far, no published data are available about the incidence of these kinds of problems in pet animals. By contrast, in human medicine and also in laboratory animals, the psychological effects of exogenous corticosteroids have been described in several studies. In the early 1950s, Brody (1952) suggested that psychiatric reactions of patients under corticosteroid treatment reflected an extreme version of a patient's usual stress reaction and recent studies report that exogenous corticosteroids can have side effects such as anxiety, depression, and increased aggression (de Kloet et al., 1999; Brown and Chandler, 2001; Hall et al., 2003). In several medical reports, a serious reaction to exogenous corticosteroids called "steroid psychosis" has been described. Steroid psychosis has been defined as a condition characterized by a series of severe symptoms, such as delirium, mania/hypomania, confusion, insomnia, memory, and attention impairments, and the incidence of such conditions seems to be related to corticosteroid dose (Wolkowitz et al., 2009). Cerebral changes and anxiety induced by prednisone have been described in rats (Gonzalez-Perez et al., 2001) as has cognitive dysfunction, with learning and memory impairment (Ramos-Remus et al., 2002). Considering the wide use of corticosteroids in veterinary medicine, it is perhaps surprising that there is lack of data and awareness relating to potential behavioral side effects, especially because the role of stress hormones in the onset of behavior problems is widely recognized in the veterinary literature (e.g., Overall, 1997; Pageat, 1998). Data about the incidence of behavioral side effects in dogs might be useful for owners to prevent them from inadvertently increasing personal risk to themselves or others when their dogs are under treatment with these drugs. To investigate this issue, our approach has been to first investigate which behaviors or changes in behavior, if any, are reported by owners after the administration of exogenous corticosteroids to their dogs.

Using an approach similar to that used in the investigation of behavioral changes associated with chronic pain in dogs (Wiseman et al., 2001), it was hypothesized that owners, through direct observations of their dogs' behavior during corticosteroid therapy, might provide a useful starting point for the development of further objective investigation. In this article, we report the first stage of an ongoing investigation into the behavioral side effects of exogenous corticosteroids in dogs. Our aim was to establish a list of possible behavioral side effects using a systematic owner interview procedure.

Materials and methods

Subjects used for this study included a self-selected convenience sample of dog owners who were recruited from

clients of veterinary clinics in the north of Italy. Inclusion criteria were that dogs had received therapy with corticosteroids for a minimum of 2 weeks in the past 6 months before the recruitment. Semi-structured interviews were conducted, in which owners were asked whether they had noticed any change in their dogs' behavior during the time they were given corticosteroids. At the beginning of the interview, owners were free to answer without any prompts, then they were prompted with questions about areas of behavior in which, on the basis of the previously published data, changes might be expected. Table 1 lists the investigated domains. The interviews terminated when data redundancy occurred. The point of data redundancy was determined as the point at which owner interviews failed to generate new information for 10 successive interviews (Sandelowski, 1995; Strauss and Corbin, 1998).

The owners of 31 mixed-breed dogs (each owner had only 1 dog enrolled in the study), 19 male dogs and 12 females of different ages (range of ages from 1 to 13 years), were included in the case series. Of 31 dogs, 18 had been administered methylprednisolone (dose range, 0.2-1 mg/kg), 8 were administered prednisolone (dose range, 0.2-1 mg/kg), and 5 dexamethasone (dose range, 0.01-0.3). Further details concerning individual subjects and the dosing procedure used are given in Table 2. Of these 31 dogs, 9 were also receiving antibiotic therapy. In all, 24 dogs suffered from a dermatological condition, 5 from arthritis, 1 from myasthenia gravis, and 1 from recurrent otitis (Table 2).

Results

In all, 11 owners reported that they considered there were behavioral changes in their dogs during corticosteroid therapy. Nine owners reported that their dogs showed more than one behavioral change, and of these 9 dogs, 2 were also receiving treatment with antibiotics (amoxicillin and clavulanic acid). Six dogs reportedly showed nervousness and/or restlessness, 3 an increase in startle responses, 3 food guarding, 2 a decrease in their activity level, 3 an increase of avoidance responses, 4 irritable aggression, and 2 increased barking. These interpretations were deduced and summarized from the owners' descriptions and were represented by the onset of certain behaviors or by an increase in their frequency and/or intensity. The findings are summarized in Table 3.

Brief case reports on cases showing behavioral changes during treatment are given later in the text.

The owner of dog 1 reported that after a few days of corticosteroid therapy the dog became more aggressive in the presence of food and in general when disturbed or approached. The owner of dog 3 described the dog as a very sweet and calm dog before the therapy, whereas after the second injection of dexamethasone (day 3 of therapy), the dog became restless, very nervous, and tended to be startled by even minimal sound. The owner reported that his dog

Table 1 Areas of dog behavior that were investigated through open questions. Questions focused on differences on and off corticosteroid therapy

Question	Investigated domains	Question examples
1	General question	Did you notice any change in your dog's behavior during corticosteroid therapy? Can you describe this change?
2	Dog personality	How would you describe your dog's personality? Is this changed in any way during corticosteroid therapy? Can you describe this change?
3	Behavior with family members	Have you noticed any change towards family members when the dog was under corticosteroid therapy? Can you describe this change?
4	Behavior with strangers at home and outside	Have you noticed any change towards strangers or guests at home or people outside, when the dog was under corticosteroid therapy? Can you describe this change?
5	Behavior when left alone	Have you noticed any change in your dog's behavior when he/she was left alone at home, during corticosteroid therapy? Can you describe this change?
6	Behavior during walks	Have you noticed any change in your dog's behavior during walks when the dog was under corticosteroid therapy? Can you describe this change?
7	Fears and avoidance behaviors	Have you noticed any change as far as fearfulness or avoidance of people, animals or situations when the dog was under corticosteroid therapy? Can you describe this change?
8	Barking	Have you noticed any change in the intensity of barking in general, when the dog was under corticosteroid therapy? Can you describe this change?
9	Eating	Have you noticed any change in behavior around food when the dog was under corticosteroid therapy? Can you describe this change?
10	Drinking	Have you noticed any change related drinking when the dog was under corticosteroid therapy? Can you describe this change?
11	Sleeping	Have you noticed any change in the way your dog sleeps when the dog was under corticosteroid therapy? Can you describe this change?
12	Behavior with other animals	Have you noticed any change in behavior towards other dogs or other animals when the dog was under corticosteroids? Can you describe this change?
13	Behavior problems	Have you noticed any behavior problem that was not present before, when the dog was under corticosteroid therapy? Can you describe this change?

was almost impossible to keep calm, but that after a few days following the interruption of corticosteroid treatment the dog gradually returned to his usual behavior and reactivity level.

The owner of dog 7 also reported that after a few days of therapy the dog was more prone to startling at every sound and stimulus but was less active, in general, during the day, while appearing restless in the evening.

Dog 10 received 5 mg of prednisone (0.3 mg/kg) for 2 months and the owner described that after a few days of therapy, the dog started to show aggression in the presence of food and became very difficult to manage because he had become very nervous, restless, and showed increased attention-seeking by barking and jumping on people.

When the therapy was discontinued, the dog gradually became more calm and manageable.

The owners of dogs 12 and 15 both described that their dogs tended to stay isolated from social contexts, in particular when people spoke loudly.

Dog 12 had been rescued when she was 2 years old and the owner described the dog as fearful and tending to avoid people when first obtained, although this resolved with time. After corticosteroid treatment, the owner reported that the dog seemed to have returned to showing the behavior she had expressed at the time she was adopted, several years before. Both these dogs (dogs 12 and 15) reportedly returned to their more usual behavior when therapy was discontinued.

Table 2 Breed, gender and reproductive status, age, disease, type, and doses of administered corticosteroids of the 31 dogs included in the study

Dog	Dog breed/type	Age (years)	Gender	Medical condition	Corticosteroid	SD mg/kg	W/MD mg/kg	Concomitant drug therapies
1	Charplanina sheep dog	5	M	Dermatological	Methylprednisolone	0.6	0.3	Antibiotics
2	Crossbreed	3	F	Dermatological	Methylprednisolone	0.5	0.25	
3	Crossbreed	8	M	Arthritis	Dexamethazone	0.15	0.07	
4	Jack russel terrier	2	F	Dermatological	Methylprednisolone	0.5	0.25	Antibiotics
5	Pomeranian	4	M	Dermatological	Methylprednisolone	0.6	0.30	
6	Fox terrier	6	M	Dermatological	Prednisolone	0.5	0.25	
7	WHWT	9	FS	Dermatological	Prednisolone	0.6	0.3	Antibiotics
8	Crossbreed	3	FS	Dermatological	Methylprednisolone	0.5	0.25	
9	crossbreed	7	M	Dermatological	Methylprednisolone	1.0	0.5	
10	Beagle	5	M	Dermatological	Prednisolone	0.3	0.3	Antibiotics
11	German shepherd	2	M	Dermatological	Methylprednisolone	0.4	0.2	
12	Italian Hound	7	FS	Dermatological	Methylprednisolone	0.6	0.3	
13	Crossbreed	3	M	Dermatological	Methylprednisolone	0.5	0.25	Antibiotics
14	Golden retriever	8	FS	Dermatological	Prednisolone	0.4	0.2	
15	WHWT	13	FS	Dermatological	Methylprednisolone	0.5	0.25	
16	Boxer	1	M	Dermatological	Methylprednisolone	1.0	0.5	Antibiotics
17	Crossbreed	6	M	Dermatological	Prednisolone	0.5	0.25	
18	Miniature schnautzer	11	F	Arthritis	Dexamethazone	0.3	0.15	
19	Yorkshire terrier	12	M	Recurrent otitis	Prednisolone	0.4	0.2	Antibiotics
20	Dachshund	3	M	Dermatological	Methylprednisolone	0.4	0.2	
21	German shepherd	2	F	Dermatological	Methylprednisolone	0.5	0.25	
22	Bull terrier	2	F	Dermatological	Methylprednisolone	0.6	0.3	Antibiotics
23	Crossbreed	7	M	Dermatological	Methylprednisolone	0.5	0.25	
24	Lagotto Romagnolo	6	M	Myasthenia gravis	Prednisolone	0.5	0.25	
25	Golden Retriever	4	M	Dermatological	Methylprednisolone	0.6	0.3	Antibiotics
26	Bernese mountain dog	8	F	Arthritis	Dexamethazone	0.2	0.1	
27	German shepherd	9	M	Arthritis	Dexamethazone	0.1	0.05	
28	Boxer	9	F	Arthritis	Dexamethazone	0.06	0.03	Antibiotics
29	Crossbreed	5	M	Dermatological	Methylprednisolone	0.5	0.25	
30	Maremmano abruzzese shepherd	4	M	Dermatological	Prednisolone	0.4	0.2	
31	Bergamasco shepherd	8	M	Dermatological	Methylprednisolone	0.8	0.4	

WHWT, West Highland White Terrier; M, male; F, female; FS, spayed female; SD, starting dose of corticosteroids; WD/MD, weaning dose/maintenance dose of corticosteroids.

Table 3 Breed, age, gender, medical condition, concomitant therapies, and behavioral changes reported in 11 dogs with identified changes during corticosteroid therapies^a

Dog	Dog breed	Age	Gender	Medical condition	Corticosteroid	Concomitant drug therapies	Reported behavioral changes
1	Charlania sheep dog	5	M	Dermatological	Methylprednisolone	Antibiotics (amoxicillin and clavulanic acid)	Food guarding; irritable aggression
3	Crossbreed	8	M	Arthritic	Dexamethazone	None	Nervousness; increased startle response
7	WHWT	9	F	Dermatological	Prednisolone	None	Nervousness and decreased activity level
10	Beagle	5	M	Dermatological	Prednisolone	None	Nervousness; food guarding
12	Italian hound	7	F	Dermatological	Methylprednisolone	None	Nervousness; onset/increased avoidance behaviors
15	WHWT	13	F	Dermatological	Methylprednisolone	None	Avoidance behaviors
16	Boxer	1	M	Dermatological	Methylprednisolone	None	Decreased activity level
20	Dachshund	3	M	Dermatological	Methylprednisolone	None	Increased startle response; increased barking
25	Golden retriever	4	M	Dermatological	Methylprednisolone	Antibiotics (amoxicillin and clavulanic acid)	Food guarding; irritable aggression
29	Crossbreed	5	M	Dermatological	Methylprednisolone	None	Nervousness; increased startle response; avoidance behaviors; increased barking
30	Maremmano abruzzese shepherd	4	M	Dermatological	Prednisolone	None	Nervousness; irritable aggression

^aDog number corresponds to number in Table 2.

Dog 16 received 1 mg of methylprednisolone on the first day of therapy. Almost immediately the owner noticed that he became very calm. The owner reported that this dog used to be very lively and jumped on people all the time. Under corticosteroid therapy he seemed to be almost sedated. The dose was reduced to half (0.5 mg/kg) on the second day of therapy and the dog appeared less sedated but still very calm until the end of the entire period of corticosteroid therapy, when he gradually returned to his previous behavior.

Dog 20 reportedly became more reactive and started to bark very frequently and intensely at even minor stimuli a few days after the beginning of corticosteroid therapy. During the second week, the owner interrupted the therapy and the dog apparently became gradually calmer and returned to his previous behavior. The signs of the dog's dermatological condition which preceded and followed the corticosteroid therapy and the behavior change had not been resolved at the time of interview.

The owner of dog 25 reported that during the first week of corticosteroid therapy, the dog started to growl at anyone who came close to his food bowl and also bit a family person who tried to pet the dog while he was lying on his bed. The dog had started to suffer from the dermatological problem some days before the onset of the therapy, but did not apparently show any sign of aggression until the initiation of the therapy. When corticosteroids were discontinued, the dog stopped growling in similar contexts.

The owner of dog 29 reported that after a few days of treatment the dog started to become nervous and agitated, and to bark incessantly at almost any stimulus, to the point that they could not leave him in the garden anymore. The dog also started to react fearfully toward people during walks and to bark at them. Although the dermatological problem was not resolved, they decided to interrupt the therapy in the third week of treatment and the dog gradually returned to his previous behavior.

The owner of dog 30 reported that, after a few days of therapy, he started to become nervous and showed aggression toward both strangers and family members for no apparent reason beyond coming close to him. These episodes happened during the second week of therapy while the dog was taking half of the starting dose of methylprednisolone. The owner decided to interrupt the therapy and to treat the dog just with antibiotics. After the interruption of corticosteroid therapy, the dog seemed to return to its previous behavior but the dermatological condition worsened. After 1 month, the veterinary surgeon suggested starting corticosteroids again, at the dose of 0.3 mg/kg of prednisolone. After a few days, the dog began to become nervous again and bit the owner on his arm when he tried to hold him.

Discussion

The findings of this descriptive analysis of a series of cases in which dogs have been treated with corticosteroids seem

to suggest that, at least in some cases, behavioral changes may occur subsequent to treatment, and that this is a potentially valuable subject for further study. However, the findings reported should be interpreted with caution for several reasons. As discussed later, it is difficult to establish from these cases the extent to which any behavioral changes are associated with corticosteroid therapy rather than changes in the signs or severity of the underlying disease being treated. In addition, without a comparative control group, it is not possible to determine the extent to which owners may report behavioral changes when asked to recall them without any therapeutic intervention.

The method of collecting data through the reports of care takers, in a manner similar to that used in this study, has been employed in several studies, both in human and in nonhuman animals (Hall et al., 2003; Wells, 2009). It has been argued that because owners spend considerable time in contact with their animals, they are in the best position to provide interpretive information on the overall patterns of behavior of their own dogs (Wemelsfelder, 1997; Wiseman et al., 2001). However, in this case both the method of recruitment of owners and the style of questioning used are likely to have increased the reporting of behavioral changes: the former as owners with concerns about corticosteroid treatment may have been more likely to enroll, and the latter because asking owners directly about changes in behavior associated with corticosteroid therapy is likely to increase the chance that such behaviors are reported. In addition, owners were asked to recall retrospectively about the behavior of their pet before and during treatment, after a variable period, thereby making their responses susceptible to variable recall biases.

Although findings are descriptive and should be interpreted with caution, some of the behaviors described by owners are consistent with the predictions which might be made from scientific reports in other species, which might suggest a general increased reaction to stimuli, especially potentially aversive ones. Nonetheless, it must be recognized that other factors in the study population might be important for the apparent onset of behavioral changes. For example, the stress and discomfort associated with conditions, such as pruritus and pain, that necessitate the use of glucocorticoid therapies might alter the behavior of the animals (Seksel, 2007). If a subject is stressed either physiologically or psychologically, adrenocorticotrophic hormone production is enhanced, regardless of the levels of circulating cortisol, and thus it might be expected that the behavioral symptomatology of dogs that already have some form of behavioral disturbance may be exacerbated by treatment, rather than being created by the exogenous steroid treatment (McEwen, 2000). In this article, the main goal was to find items for future investigation through a controlled survey and therefore owners were prompted to use their own words to describe their dogs' behaviors in an open and articulate way. Among the cases in which behavioral changes were reported by owners, most owners

spontaneously reported several important behavioral changes after the first general questions (Table 1, Q1 and Q2). These questions could easily be included in routine follow-up monitoring in practice and may help alert practitioners to potential risks they need to be aware of, especially given that in several cases, treatment was associated with aggression and an actual bite in 2 incidences.

Most of the dogs involved in the study suffered from dermatological problems and were treated with oral corticosteroids, methylprednisolone, and prednisolone, with similar dose ranges and therapeutic schedules, starting with a higher dose in the first week of therapy and continuing with half dosage for another week and, finally with the same half dosage every 2 days for variable periods. It is noteworthy that the reported behavioral changes seemed to appear quite early during corticosteroid treatments and this seems to be consistent with data reported in human studies (Warrington and Bostwick, 2006). Two dog owners (dog 20 and dog 29) explicitly reported a stronger association with corticosteroid treatment rather than the clinical condition for which the medication was prescribed, and this might be important as the latter could be a confounding factor in many case reports. Nonetheless, it must be acknowledged at this time that owners may easily perceive relationships when none actually exist, especially because some may perceive corticosteroids as "dangerous" drugs (Cullen et al., 2006).

The coadministration of other therapies further complicates the interpretation of the results. It has been shown that some antibiotics can affect behavior, for example penicillin and its analogues have been associated with sedation and anxiety, whereas quinolones, widely used in dogs and cats might cause, in rare cases, restlessness and irritability or, on the contrary, lethargy (Sternbach and State, 1997; Turjanski and Lloyd, 2005). Other drugs that are often used in dogs, such as topical or oral antiparasitic products, can have effects on behavior (Florio et al., 1989; de Souza Spinoza et al., 2000).

It is reasonable to suggest that exogenous corticosteroid dosage might influence the onset of behavioral side effects and, in the present study sample, dose ranges varied and questions were directed generally to obtain information of changes in behavior on and off therapy, without any specific investigation about dose-related effects. Dose and type of corticosteroid used should be more thoroughly considered in future studies, along with other elements related to the pharmacokinetics of these drugs; for example, it has been reported that unbound serum prednisolone levels are higher during periods of hypoalbuminemia (Wolkowitz et al., 1990). Investigating the effect of corticosteroid dose is a particularly challenging issue because they are often prescribed in dogs with changing titrations and are adjusted according to the desired effect. A more specific investigation at different dosages would be useful to discern any possible correlation, as has been reported in human beings, between dose and behavioral disturbances, although even the human data are not consistent (Hall et al., 2003; Felder-Puig et al., 2007).

Other factors to consider include genetic influences on negative feedback mechanisms that are involved in the stress response (Gomez et al., 1998) and the rhythmicity of hypothalamic pituitary adrenal axis activity. Cortisol is secreted in a pulsatory manner; circadian patterns, similar to the ones that have been demonstrated in human beings, are difficult to verify in dogs probably because of the very different housing and management conditions of individual dogs and groups of dogs (in shelters, laboratories, in breeder kennels in households, etc.), but it seems reasonable to suggest that there are patterns of diurnal activity, depending on dog management conditions (Kolevská et al., 2003). Feeding seems to be one of the most important regulatory elements for the synchronization of HPA activity (Leal and Moreira, 1997). Exogenous corticosteroid might therefore interfere with the normal physiology of HPA axis depending on the management of dogs and we cannot exclude that some effects were influenced by external factors such as the time of feeding or the housing conditions. The effects might also be indirect; for example, the appetite stimulant effects of corticosteroids are widely recognized, and if a dog is hungrier, it may be more prone to guard food and show aggression. Other indirect relationships might also exist with some of the other reported signs.

The aforementioned issues suggest caution in the interpretation of these results, but we nonetheless suggest that they should encourage further investigation from many perspectives of this potentially important issue, given both the strong theoretical basis for corticosteroids increasing vigilance and biasing sensitivity toward aversion. Considering the lack of evidence to the contrary, we further suggest, at this time, that veterinarians should not only monitor for behavioral side effects but also consider offering some precautionary behavioral management advice to owners when dispensing these drugs.

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Behavioural changes in dogs treated with corticosteroids



Lorella Notari, Oliver Burman, Daniel Mills

Animal Behaviour, Cognition and Welfare Group, School of Life Sciences, Joseph Banks Laboratories, University of Lincoln, Lincoln LN6 7DL, UK

HIGHLIGHTS

- We aimed to identify behavioural changes in dogs treated with corticosteroids.
- Dogs on corticosteroids showed behaviour associated with a negative affective state.
- In a behavioural test, dogs on corticosteroids avoided a mildly aversive stimulus.
- Dog owners should be advised by veterinarians about behavioural risk management.

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ABSTRACT

In human medicine, psychiatric side effects among patients on corticosteroid therapy are widely reported, but this appears to have been largely overlooked in the animal literature despite glucocorticoids being widely used in veterinary medicine. Therefore the aim of the current study was to identify possible psycho-behavioural changes in dogs treated with corticosteroids. Two different methodologies were used. Firstly, dog owners were asked to fill a 12 item questionnaire aimed at further validating the initial results of a previous survey relating to changes seen when their dog was receiving corticosteroid treatment. In a second study, a population of dogs undertook behavioural tests aimed at objectively identifying changes when receiving corticosteroid therapy.

In the first study, a sample of owners whose dogs were receiving treatment for dermatological, orthopaedic or other conditions evaluated their dogs' behaviour on and off therapy, using a seven point scale. The survey was completed by 44 dog owners with dogs receiving treatment with a range of corticosteroid preparations (mainly prednisolone and methylprednisolone) and 54 dog owners with dogs receiving treatment with other drugs, mainly antibiotics and non-steroidal anti-inflammatory drugs. Dogs under corticosteroid treatment were reported to be significantly less playful, more nervous/restless, more fearful/less confident, more aggressive in the presence of food, more prone to barking, more prone to startle, more prone to reacting aggressively when disturbed, and more prone to avoiding people or unusual situations.

In the second study, eleven "treatment" dogs were tested both before and during corticosteroid treatment with either methyl-prednisolone or prednisolone to assess their sensitivity to a potentially aversive sound stimulus. Eleven control dogs were also tested at the same time intervals in the same environment. Dogs were exposed to a brief dog growl while they explored bowls containing food and their behaviour was video recorded. Treatment dogs were found to investigate the area in the vicinity of the bowls for significantly less time and to eat significantly fewer pieces of food when on corticosteroids, compared to control dogs, after hearing the growl. These results provide the first empirical evidence of possible adverse psycho-behavioural side effects in a veterinary clinical setting following the use of corticosteroids, and suggest the need for concomitant behavioural advice when these drugs are used in general veterinary practise to avoid the risks associated with these changes.

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1. Introduction

Glucocorticoids are widely used in veterinary practice but are also among the most important mediators of the stress-response [1,2]. This response has physiological, behavioural, cognitive and

emotional components, having the potential to inhibit positively motivated responses and to increase anxiety-related behaviours [3–5]. Glucocorticoids mediate changes in cognition, learning and emotional processes through the activation of glucocorticoid receptors in diverse brain areas from the prefrontal cortex through to the hippocampus, basal ganglia and amygdale [3]. Both excesses and deficits in glucocorticoid can lead to impairment of learned and emotional processes and responses [6].

E-mail address: lorellanotari@lorellanotari.it (L. Notari).

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The therapeutic use of glucocorticoid drugs in companion animals is associated with several well recognized physical side effects including gastro-intestinal problems, suppression of adrenal gland function and increased risk of infections [7,8]; possible psychological side effects have not received much attention beyond a preliminary survey reported by the authors [9]. However, in human medicine, several surveys and case reports have shown that important psychiatric side effects can occur in patients on corticosteroid therapy. It has been suggested that the onset of corticosteroid-induced psychiatric disturbances might be linked to pre-existing individual psychological characteristics such as personality, with these reactions reflecting an extreme version of a patient's usual stress reaction [10–12]. Increasing attention is being given to these effects in human patients, receiving long term treatment [11,13,14]. Neurological toxicity due to the drug itself or the synergistic action of drugs administered concurrently have been postulated for the unexpected behavioural and psychiatric effects of these medications when prescribed for physical diseases [5], these effects are probably most often related to the neurochemical cascades linked to the stress response [14–16].

Alongside studies in human medicine, there is abundant evidence of the influence of exogenous pituitary adrenal hormones on animal behaviour but not specifically in a therapeutic context as might occur in companion animals. Studies in laboratory animals have shown that exogenous glucocorticoids can affect cognitive functions such as learning and memory [6,17–19]. An effect on the emotional states of animals has also been hypothesized with, for example, the experimental administration of corticosteroids to rats appearing to influence their subsequent emotional response to unexpected reductions in reward size [20]. This study showed that rats treated with corticosteroids responded to an unexpected downshift in reward magnitude by showing a significantly greater decrease in their consummatory behaviour – interpreted as an expression of their emotional response – compared to a control group subjected to the same procedure. Many of the behavioural effects of corticosteroids would be expected if these chemicals induced a negative cognitive bias, e.g. a greater sensitivity to potentially threatening stimuli in the environment [21].

Investigating the potential negative behavioural side effects of glucocorticoid drugs in companion animals is clearly important in order to make a full risk–benefit analysis concerning their use, and to ensure that appropriate advice can be given to owners and veterinarians when these drugs are prescribed. Therefore the present study aimed to investigate the effects of corticosteroid therapies on dog behaviour:

firstly, through a retrospective study using questionnaire responses concerning the behaviour of dogs when on and off corticosteroid therapy; and secondly, through a case–control study of the responses of subjects in a behavioural test aimed at assessing the animal's response to a potentially threatening sound stimulus.

2. Materials and methods

2.1. Questionnaire study

A 12 item questionnaire was completed by dog owners with dogs receiving or having recently received drug treatment, preferably for dermatological or orthopaedic conditions. The questionnaire was informed by the results of a previous survey [9]. Seven of the 12 items were selected on the basis of the results of a previous survey [9], with five further questions ('fillers') relating to other behavioural changes not identified in the previous survey selected among behaviours that frequently cause complaints by dog owners [22,23], but not thought to be influenced by corticosteroids. These were inserted partly as 'fillers' and to aid validation of target effects [24]. The questionnaire was published via the Internet in both English and Italian, with a paper version also distributed to Italian veterinary clinic clients. Questionnaires were back translated by independent mother tongue translators to assess the consistency of the two versions. The items were scored on a seven point scale with two scores for each question posed: one score for the respondent's perception when the animal was receiving pharmacological treatment for the condition and one for when the animal was not receiving pharmacological treatment (Fig. 1).

The introductory part of the questionnaire gathered demographic data relating to both the owner and their dog, information about the drugs being given to the dog at the time of survey (such as type of drug, time of administration and doses) and information about the type of condition/disease for which it was being used. The respondents were asked to mention all drugs taken in the same period for the same or other concomitant conditions. The questionnaire was to be completed on the Internet and advertised through veterinary associations, pet websites and magazines both in Italy and UK. Paper questionnaires were also distributed in veterinary clinics in the north of Italy.

Questions are illustrated in Table 1 and Items 1 (Play behaviour), 5 (Attention seeking), 7 (Obedience), 8 (Guarding behaviour) and 12 (Mounting behaviour) were added as additional fillers.

WHEN YOU ANSWER THE FOLLOWING QUESTIONS, YOU ARE KINDLY REQUESTED TO THINK, AT FIRST, ABOUT THE BEHAVIOUR OF YOUR DOG BEFORE STARTING THE DRUG AND PUT AN X WHERE APPROPRIATE IN THE UPPER UNSHADED STRIP. THEN YOU SHOULD THINK ABOUT YOUR DOG'S BEHAVIOUR WHILE YOUR DOG IS TAKING THE DRUG AND PUT AN X WHERE APPROPRIATE IN THE DARKER, LOWER STRIP

Q1. Play behaviour. Some dogs are very motivated to play with people, other dogs or toys. On a scale from 1 to 7 where 1 is 'not very playful' and 7 is 'very playful' how would you rate your dog's behaviour?

Not very playful ← → Very playful

Without drug							
With drug							
	1	2	3	4	5	6	7

Fig. 1. Example of question and scoring system used in owner questionnaire: Q1 Play Behaviour.

Appendix H

Table 1

Owner questionnaire. Verbatim of questions. Scales 1–7 illustrate the level of expression of the investigated behaviour.

Question numbers	Details of questions
Q1	Some dogs are very motivated to play with people, other dogs or toys. On a scale from 1 to 7 where 1 is 'not very playful' and 7 is 'very playful' how would you rate your dog's behaviour?
Q2 ^a	Thinking about your dog's temperament, how would you define its nervousness/restlessness on a scale from 1 to 7 where 1 is 'very nervous and restless' and 7 is 'very calm'?
Q3 ^a	Thinking about your dog's general responses, for example, in the presence of unknown people or of new, unknown stimuli (sounds, loud voices, unknown contexts, unknown animals or children...), on a scale from 1 to 7 where 1 is 'extremely fearful and insecure' and 7 is 'very confident', how would you rate your dog?
Q4	Thinking about your dog's behaviour when there is food around, on a scale from 1 to 7 where 1 is not at all aggressive and 7 is very aggressive in the presence of food, how would you rate your dog?
Q5	Some dogs tend to be very insistent and seek physical contact with owners by jumping up, snapping, scratching with a front paw, whining or barking: on a scale from 1 to 7 where 1 is 'no attention seeking behaviours' and 7 is 'frequent and intense attention seeking behaviours', how would you rate your dog?
Q6	Some dogs bark at any time, night and day, some others bark only in exceptional occasions. On a scale from 1 to 7 where 1 is 'rare barking' and 7 is 'frequent and intense barking', how would you rate your dog's behaviour?
Q7	Some dogs are very obedient, for example they come when called and go to bed when asked, while some others are less easily controlled. On a scale from 1 to 7 where 1 is 'not at all obedient' and 7 is 'very obedient', how would you rate your dog's behaviour?
Q8	Some dogs are very predisposed to guarding behaviour and tend to threaten people by barking and growling, some others are friendly with everyone and don't show any guarding behaviour. On a scale from 1 to 7 where 1 is 'no guarding behaviour' and 7 is 'intense & frequent guarding behaviour', how would you define your dog's behaviour?
Q9	Some dogs tend to startle very easy, for example when they hear a sound or are suddenly touched. In these cases they can react by fleeing, getting jumpy or showing aggression. On a scale from 1 to 7 where 1 is 'low/rare startle response' and 7 is an 'excessive and very frequent startle response', how would you define your dog's behaviour?
Q10	Some dogs tend to react aggressively if someone tries to touch them or come close while they are resting. These dogs can become aggressive whenever the owner tries to brush them, medicate them or even simply tries to pet them. On a scale from 1 to 7 where 1 is 'never aggressive when disturbed/restrained' and 7 is 'very aggressive when disturbed/restrained', how would you define your dog's behaviour?
Q11	Some dogs have a marked tendency to avoid people or situations that are unknown or unfamiliar, for example they tend to leave the room when unknown guests arrive or when people scream or there are loud noises. On a scale from 1 to 7 where 1 is 'no tendency to hide or avoid people or situations' and 7 is 'High tendency to hide or avoid people or situations', how would you rate your dog's behaviour?
Q12	Some dogs can show a tendency to mount people (children and adults) or other dogs, often of the same sex. On a scale from 1 to 7 where 1 is 'no tendency to mount' and 7 is 'high tendency to mount' how would you rate your dog's behaviour?

^a Reversed scale.

Responses were collated and analysed using a repeated measures multivariate GLM (SPSS 21). In this analysis treatment related effects on behaviour when on and off drug were considered as dependent measures; types of treatment (divided into 3 categories: corticosteroids, corticosteroids and other drugs, only drugs other than corticosteroids), duration of treatment (divided into 5 categories: 1 week, 1–2 weeks, 2–3 weeks, 2–4 weeks, more than 4 weeks), the reason for treatment (divided into 3 categories: dermatological conditions, orthopaedic conditions and others) were considered independent factors. This first multivariate analysis was made to test drug effects within-subjects, and since only treatment type was found to be a significant factor and there was great variation in the baseline value of subjects, a univariate GLM was then used to examine the difference between behaviour when on and off treatment versus treatment type,

with post hoc comparisons corrected for multiple testing by means of a Bonferroni correction procedure.

2.2. Behavioural test

2.2.1. Subjects

Eleven dogs receiving (or due to receive) corticosteroid treatment and 11 control dogs were recruited and successfully completed two sessions of the behavioural test. Treatment dogs were recruited from the patients of veterinary practices in the North of Italy. Veterinarians were asked to propose dog owners that had received prescriptions of oral corticosteroid drugs for dermatological problems to participate in the research. Criteria for inclusion were that dogs had not been prescribed any other medication; the prescription dose range was within 0.4–0.5 mg/kg of prednisone or methylprednisolone every day. Control dogs were recruited from among the healthy patients of veterinary practices and clients of dog trainers. Control dogs were tested twice in the same environment as the dogs on corticosteroids, with the same time interval between the two tests. Details of all subjects are given in Table 2.

The first behavioural test for treatment dogs occurred just before they started therapy, with a second taking place 6–7 days into the therapy, often just before the dose of corticosteroid started being reduced with a view to its withdrawal.

2.3. Test procedure

The tests were conducted in three different locations in order to accommodate the travel restrictions of clients, but the set-up was the same at each of these: a room of sufficient size to accommodate the experimental apparatus, with a chair for the owner at the opposite end of the room. The apparatus composed of a screen covered with a blanket that hid a loudspeaker system connected to a computer. Five pots were placed in front of the screen, 35 cm from the loudspeakers. The pots were placed in a way that enabled the researcher to put small pieces of food into them at the same time (Fig. 2). For each dog, the kind of treat used during testing was indicated by the owner as being the dog's favourite. The same kind of treat was used in both test trials for that subject. Two video cameras (Canon Legria HF R506) were used to record the dog's behaviour during testing for later

Table 2

Dogs involved in the study. M = male dog; F = female.

Dog n.	Breed/type	Gender	Age	Treatment ^a
1	Collie	M (n)	9	0.5/kg prednisone
2	Dachshund	M	4	0.4/kg prednisone
3	Labrador	M	5	0.4/kg methylprednisolone
4	Golden retriever	F (n)	10	0.5/kg prednisone
5	Crossbreed	F (n)	4	0.4/kg prednisone
6	Cocker	M	9	0.4/kg methylprednisolone
7	Pitbull	M (n)	7	0.4/kg prednisone
8	Jack Russell	M	3	0.5/kg prednisone
9	Crossbreed	M	1	0.4/kg prednisone
10	Golden retriever	F	2	0.5/kg prednisone
11	Crossbreed	M	4	0.4/kg prednisone
12	Crossbreed	M (n)	9	No treatment
13	Crossbreed	F (n)	2	No treatment
14	Crossbreed	M	2	No treatment
15	Pitbull	M (n)	2	No treatment
16	Shiba Inu	M	1	No treatment
17	German shepherd	F	1	No treatment
18	Crossbreed	F (n)	7	No treatment
19	Cocker Spaniel	M	6	No treatment
20	Border collie	M	5	No treatment
21	German shepherd	M	4	No treatment
22	Crossbreed	F (n)	1	No treatment

Dog (n) = neutered.

^a No dogs were receiving treatment in the first test trial, treatment refers to medication in use during the second test trial.

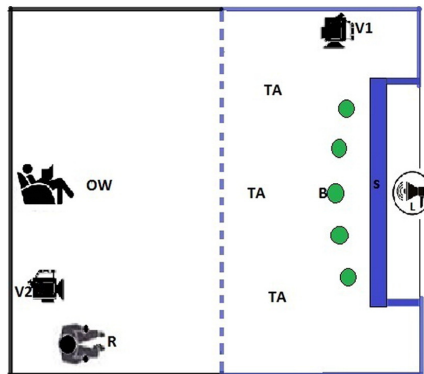


Fig. 2. Test setting. V1 and V2 = video cameras; TA = test area; S = screen;

behavioural coding. The video cameras were mounted on a tripod, one to one side of the room and the other at the back of the room. The part of the room within 150 cm of the screen was considered to be the “test area” and when dogs were within this area with evident interest in exploring it, the screen or the pots, their behaviour was considered as ‘exploring the test area’.

Exploring the test area included:

1. Sniffing = The dog overtly approached the floor, the bowls or the screen and appeared to inhale through its nose
2. Exploring = Remaining in the test area watching towards the floor, the screen or the bowls
3. Investigating the pots = approaching the pots with nose within 1 cm of pot and nose or muzzle inside pot

Behaviours such as staying far from the screen, either close to the owner or at the opposite end of the room to the experimental apparatus, were considered as behaviours associated with not exploring the test area.

Dogs were brought into the test environment on a leash by their owners. In each test trial the owner was invited to calmly restrain the dog on the leash, sit and wear a pair of sunglasses to restrict eye contact between dog and their owner. The researcher showed the dog a few pieces of food and then put one small piece of food in each pot. After this, the researcher sat on a chair in a corner of the room, showing no overt interest in the procedure. The owner was instructed to unleash the dog and then behave in a neutral way, pretending to read a book provided by the researcher and completely ignoring the dog until a signal signifying the end of the test was given. The dog was left free to investigate the test area and take the treats from two pots. As soon as it started to approach the third pot, playback of a three second growl was started. Three types of dog growl recordings were used: small dog, medium dog and large dog growls, and these were allocated on the basis of the size of the dogs being tested (i.e. small test dog = small dog growl) in order to minimize the scaring effect of the growl. The growls were chosen because they had been recorded in the context of food guarding and used in a previous study [25]. The dog's behaviour was then observed for two minutes. At the end of the test, the owner was asked to call the dog and put it on its leash. This behavioural test procedure is illustrated in Fig. 3.

B = pot disposition; L = loudspeaker; OW = owner position; R = researcher position during the test.

2.4. Behavioural observation

The video recordings of tests were analysed using Solomon Coder (<http://solomoncoder.com/>). We considered the following behaviours for analysis:

1. Latency
 - a. Time from release to the approach to the first pot (nose within 1 cm of pot) (Latency 1)
 - b. Time from the growl/startle reaction to further investigation of pots (Latency 2)
2. Time spent investigating the test area (TTA)
3. Time spent investigating the pots (TTP)
4. Time spent investigating the test area before the growl (Expl1)

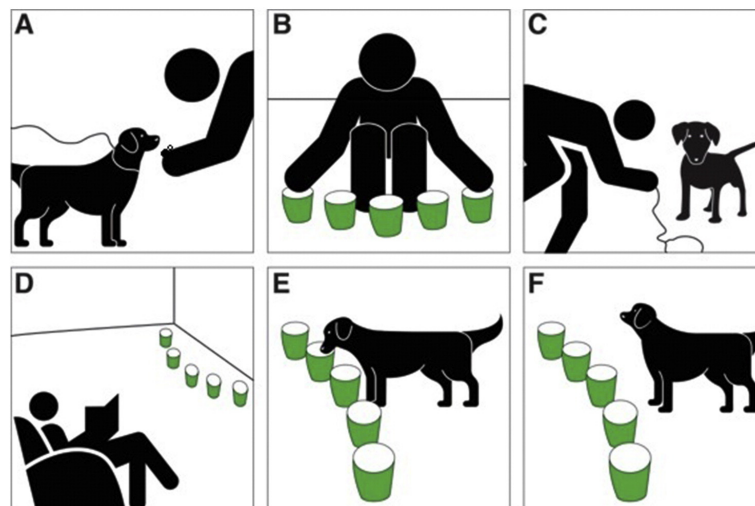


Fig. 3. Behavioural test procedure. A = the dog is shown the food; B = a piece of food is placed into each pot; C = the owner unleashes the dog; D = the owner is instructed to behave in a neutral way; E = the dog explores the pots; F = the dog reacts to the growl.

5. Time spent investigating the test area after the growl (Expl2)
6. Startle reactions
 - a. Grade 1. The dog responds with minimal, momentary re-orientation of head
 - b. Grade 2. The dog responds with re-orientation of head, steps back
 - c. Grade 3. The dog responds with re-orientation of head, steps back and takes a few seconds before coming back to the pot or leaves the test area and does not return within 2 min
7. Eating of food. The number of food pieces eaten by each dog in each test trial.

2.5. Data analysis

The first observer transcribed the video recordings of both test trials ($n = 22$) and scored them using the ethogram on two separate occasions to assess intra-observer reliability. The recordings from ten of the dogs (5 treatment dogs and 5 control dogs) were randomly selected for their behaviour to be assessed by a second observer who was 'blind' to the treatment allocation in order to evaluate inter-observer variability. Spearman's coefficient was used to measure pairwise correlation among raters.

Data from the video analysis regarding Latency 1, Latency 2, TTP, TTV, Expl1 and Expl2 were analysed using SPSS 21. Data were not normally distributed and therefore an extension of the Generalized Linear Model (GLM), Generalized Estimating Equations (GEE) was used in order to evaluate the results that accommodated correlated within-subjects data and allowed comparisons between subjects.

Startle reactions were evaluated for their severity according to the above descriptions. Eating of food was evaluated by counting the number of food pieces eaten by each dog during each test trial. Comparison between the control and treatment dogs for these two metrics was evaluated using Mann–Whitney U test at a given time point (e.g. either first or second test), with Wilcoxon's Matched Pairs Signed-Ranks Test used to compare within groups between tests (first versus second test).

3. Results

3.1. Questionnaire study

By the close of the survey in February 2011, 98 questionnaires had been completed correctly by dog owners and considered suitable for analysis. Dogs were from a variety of breeds and genders, and aged between 1–14 years. Reasons for treatment were dermatological conditions ($n = 55$), orthopaedic conditions ($n = 36$) and other kinds of condition ($n = 7$). Treatment duration varied from one week of treatment to long term maintenance treatment. Of the sample of 98 dogs, 44 received corticosteroids and 54 received only other medications, mainly antibiotics ($n = 20$) and non-steroidal anti-inflammatory drugs ($n = 28$), with a small proportion on other drugs ($n = 6$). Of the 44 dogs that received corticosteroids, 23 also received other drugs, mainly antibiotics. The 44 dogs receiving treatment with corticosteroids were subject to a variety of corticosteroid preparations, but mainly prednisone/prednisolone ($n = 32$) and methylprednisolone ($n = 7$). Two dogs received betamethasone and 3 dogs received dexamethasone. Corticosteroid drug doses were between 0.1–1.2 mg/kg for prednisone and prednisolone, between 0.5–1.5 mg/kg for methylprednisolone, 0.05 mg/kg for betamethasone and 0.1 mg/kg for dexamethasone.

Data were not normally distributed, but still suitable for analysis of variance [26,27]. The repeated measures multivariate GLM analysis showed that the only significant factor related to a change in the behaviour of dogs on and off corticosteroids was the treatment used.

The univariate GLM with post hoc correction for multiple testing showed that the administration of treatments involving corticosteroid (44 dogs) had a statistically significant effect on the response to eight

items. Five behaviours, Play ($F = 6.525$, Nervousness ($F = 6.130$), Fear ($F = 13.112$), Startle reactions ($F = 5.705$), Irritable aggression ($F = 5.080$) – all significantly changed with $p < 0.01$; three behaviours, Food aggression ($F = 4.793$), Barking ($F = 4.330$), Avoidance ($F = 4.463$) – all significantly changed with $p < 0.05$). By contrast, treatments without corticosteroids (54 dogs), produced no significant changes ($p > 0.05$) in response to any item and no significant changes in behaviour were related to other drugs (Table 3).

3.2. Behavioural test

The behavioural testing of dogs (11 sample dogs and 11 control dogs) ended in October 2013.

Spearman's coefficient of correlation revealed statistically significant positive correlations between intra-observer ($n = 22$) and inter-observer ($n = 10$) measurements. Intraobserver correlations were positive with $r = 0.994$ and $p < 0.01$. Interobserver correlations were positive with $r = 0.996$ and $p < 0.01$ for all items.

GEE revealed no significant differences in Latency1, Latency 2, TTA, TTP, EXPL1 and EXPL2 between groups in the first test trial, before the 'treatment' dogs had been placed on corticosteroids. In the second test trial, the total time spent investigating the test area (TTA) was significantly lower in the group of dogs treated with corticosteroids (unstandardized coefficient $B = 25.309$; $\chi^2(1) = 6.157$; $p < 0.05$) compared with the control group of dogs. In the second test trial, the exploration time after the growl of dogs (EXPL2) in the treatment group was significantly lower ($B = 26.18$; $\chi^2(1) = 6.600$; $p < 0.05$) compared with the same behaviour in the control group of dogs. Latency times (L1 and L2), time spent investigating the area before the growl (EXPL1) and the time spent investigating the pots (TTP) were not significantly different between the two groups ($p > 0.05$) [Latency 1: $B = -3.573$, $\chi^2(1) = 0.588$, $p = 0.443$; Latency 2: $B = -9.709$, $\chi^2(1) = 0.477$, $p = 0.490$; EXPL1: $B = -0.545$, $\chi^2(1) = 0.310$, $p = 0.577$; TTP: $B = 5.991$, $\chi^2(1) = 2.583$, $p = 0.108$].

Startle reactions in the first test trial were present in six dogs from the treatment group and nine dogs from the control group. Three dogs from the test group were graded at level 1 (S1) and four at level 2 (S2). Eight dogs from the control group were scored at level 1 (S1) and one at level 3 (S3).

In the second test trial seven dogs from the treatment group and nine dogs from the control group produced startle reactions (see Table 4). No significant differences between groups were found as far as startle reactions were concerned ($p > 0.05$).

In the first test trial, seven dogs from the treatment group ate all five food treats, two dogs did not eat any food and two dogs ate three pieces. In the first test trial, eight dogs from the control group ate all the food, two dogs did not eat any food and one dog ate three pieces. In the second test trial, five dogs from the treatment group ate all the food, two dogs ate four pieces, two dogs ate three pieces and two dogs did not eat any food. In the second test trial, all dogs from the control group ate all the food. A Mann–Whitney U test revealed a significant difference between groups as far as number of pieces of food eaten was concerned ($z = -2.765$; $p = 0.028$) with control dogs eating more than treatment dogs. Wilcoxon's Matched Pairs Signed-Ranks Test revealed no significant differences within groups in the two test trials ($p > 0.05$) for either startle or food consumption.

4. Discussion

The results reported here from both studies are consistent with each other and the preliminary findings of Notari and Mills [9] reported previously. This latter study had the main goal of providing information about the possibility that dogs receiving treatment with corticosteroids might show behavioural analogous to those reported in humans and was the starting point for the development of the present study. The survey reported here was a development of the previous methodology,

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Table 3

Reported changes in behaviour score on and off different treatments. Scales 1–7 represent the expression of the behaviour. Questions about nervousness and fear had reversed scales.

Response item		CG Off	CG On	OG Off	OG On
Play**	Mean	4.80	4.05	3.80	4.63
	(±SD)	(1.82)	(1.71)	(1.90)	(1.85)
Nervousness**	Mean	4.57	3.75	4.69	5.02
	(±SD)	(1.56)	(1.92)	(1.78)	(1.55)
Fear**	Mean	4.89	3.95	4.54	4.94
	(±SD)	(1.48)	(1.72)	(1.61)	(1.37)
Food aggression*	Mean	2.00	2.57	2.20	2.07
	(±SD)	(1.45)	(2.11)	(1.56)	(1.37)
Attention seeking	Mean	3.91	4.14	3.93	4.15
	(±SD)	(1.70)	(1.84)	(1.86)	(1.77)
Barking*	Mean	2.73	3.43	2.74	2.67
	(±SD)	(1.69)	(2.05)	(1.75)	(1.78)
Obedience	Mean	5.18	4.91	5.07	5.04
	(±SD)	(1.48)	(1.60)	(1.33)	(1.29)
Guarding	Mean	3.39	3.50	3.20	3.31
	(±SD)	(1.87)	(2.05)	(2.10)	(1.92)
Startle reactions**	Mean	2.84	3.77	3.19	3.06
	(±SD)	(1.68)	(2.07)	(1.83)	(1.62)
Irritable aggression**	Mean	1.93	2.43	2.07	1.96
	(±SD)	(1.47)	(1.83)	(1.46)	(1.30)
Avoidance*	Mean	2.27	2.73	2.19	2.11
	(±SD)	(1.80)	(2.05)	(1.48)	(1.42)
Mounting	Mean	1.80	1.75	2.02	1.80
	(±SD)	(1.29)	(1.48)	(1.56)	(1.19)

CG = corticosteroid group; OG = other group. Significant differences between groups calculated with GLM univariate analysis indicated thus * = $p < 0.05$; ** = $p < 0.01$.

using a simple scoring system to allow the harvesting of a larger data set and the inclusion of a control group. All of the behaviours that were reported to change under the influence of corticosteroid drugs in the previous study [9] were found to change significantly in the present study, adding weight to the reliability of these reported effects. Dogs under corticosteroids were reported to be more nervous/restless, more fearful/less confident, more aggressive in the presence of food, barked more, more prone to startle, more prone to react aggressively when disturbed, and more prone to avoiding people or unusual

situations. All these findings indicated these drugs might bias sensitivity towards aversion in dogs.

In addition, one further item, not previously reported, but also possibly influenced by changes in affect i.e. amount of play behaviour, was reported to be reduced under corticosteroid treatment and elevated highly under treatment with other drugs (Table 3). The additional discovery of a disparate effect on play between the two classes of pharmacological intervention could be important as the occurrence of play is considered to be a useful indicator of animal welfare, with animals reducing play when they become distressed [21,28,29]. It therefore seems that a reduction in play would be consistent with the potential negative effects of corticosteroids, but it is worth noting that there was a large increase in response to treatment with the other drugs (Table 3), and this may be where the main effect lies, i.e. an elevation in positive mood when these other interventions are used. The finding is all the more interesting as corticosteroids are widely used for their potent anti-inflammatory effects and it might be predicted that their value in relieving pain and irritation means that their use would increase playfulness as a result. However, these results suggest that their negative effects on affective state might mitigate against the predicted positive behavioural effects. It might be that corticosteroids serve to largely increase arousal rather than induce a positive affective state per se, as might be often assumed.

Four other behaviours were inserted as fillers to prevent psychological bias [24]. These fillers were selected among the behaviours that most frequently cause complaints by dog owners [22,23], that we did not expect to be effected by the use of corticosteroids. The robustness of the effects reported here are therefore further enhanced by the finding that these four items (attention seeking, obedience, guarding and mounting) did not show significant effects. Corticosteroid drug dose effect and the effects of disease on behaviour have been widely reported in humans [1,30,31], and the impact of physiological stress and health on the behaviour of veterinary species recently reviewed [32], but there is still a lack of information on the relationship between levels of circulating corticosteroid and behaviour in animals. This was not addressed in the present study but is an area for future attention.

Behavioural tests followed the survey in order to provide, for the first time, objective behavioural evidence of the effect of corticosteroid therapy on dog behaviour. Because our initial findings were interpreted to indicate that dogs on corticosteroid therapy were more avoidant, the

Table 4

Startle reactions and pieces of food eaten by dogs in the two test trials.

Dog n.	Trial 1 Startle score	Trial 2 Startle score	Trial 1 Food eaten	Trial 2 Food eaten
1 ^a	–	–	5	5
2 ^a	S1	S2	5	5
3 ^a	–	–	5	5
4 ^a	–	–	5	4
5 ^a	S2	S3	3	3
6 ^a	–	S2	5	0
7 ^a	S2	S1	0	4
8 ^a	S2	S2	0	0
9 ^a	S1	S1	3	3
10 ^a	S2	S2	5	5
11 ^a	–	–	5	5
12	S1	S1	5	5
13	–	–	5	5
14	S1	S1	5	5
15	S1	S1	5	5
16	S1	S2	0	5
17	S1	S1	5	5
18	S3	S3	3	5
19	–	–	5	5
20	S1	S1	5	5
21	S1	S1	0	5
22	S1	S1	5	5

S1 = The dog responds with minimal, momentary re-orientation of head.

S2 = The dog responds with re-orientation of head, steps back.

S3 = The dog responds with re-orientation of head, steps back and it takes a few seconds before coming back to the pot or never comes back.

Trial 1 Food = pieces of food eaten in trial 1.

Trial 2 Food = pieces of food eaten in trial 2.

^a Dogs receiving treatment with corticosteroids in the second test trial.

test was designed to stimulate exploration of the test area with minimal challenges. The introduction of a surprising, potentially negative stimulus in the form of the growl, had the purpose of testing both reactivity and avoidance tendency. Decreases in exploratory behaviour have also been associated with negative affective states [33–35], and this is likely to be the product of a negative cognitive bias associated with negative affect: when in a negative affective state the desire to seek new information is reduced and so the animal might avoid rather than explore open areas and novel stimuli [36,37]. In the behavioural tests, types of corticosteroids and drug doses were very similar within the treatment group. Although cytokines involved in the immune response might explain behavioural effects such as decreased exploratory behaviour and increased avoidance [38] reported by owners in the survey, this would not explain the results in the behavioural tests. Some dogs suffered from allergic dermatological conditions, that would produce a lot of cytokines, when they were tested off treatment and no significant differences were found between treatment and control group dogs at this time rather a significant decrease in exploratory behaviour between groups was observed only when treatment dogs were on corticosteroids.

Unlike the dogs receiving corticosteroid treatment, control dogs did not show a significant change in their exploratory behaviour, when receiving treatment. Comparisons between groups showed that the exploratory behaviour was lower when dogs were on corticosteroids. In laboratory animals, the administration of glucocorticoids after a training session seemed to influence contextual fear memories and hippocampal long term potentiation (LTP), suggesting that they may enhance contextual fear memory consolidation via enhancing hippocampal LTP [39].

In the behavioural test, we found dogs on corticosteroids ate significantly less food compared with the control group, despite increased appetite being a well-recognized side effect of corticosteroid therapy. Decreased food intake can be interpreted as part of decreased exploratory behaviour or as a sign of increased stress in itself [40].

In the behavioural tests three dogs treated with corticosteroids increased their startle reactions and one dog decreased this reaction, while in the control group just one dog increased its startle reaction and no dog decreased it. The results about startle reactions did not show any significant differences in startle reactions between the two groups ($p > 0.05$). However, in the questionnaire study dog owners reported an increase in their dogs' startling tendency when on corticosteroids and the startle response has been used as a specific response to assess a changed negative affective state in both laboratory animals and human beings [3]. This apparent discrepancy can be explained because startle responses have different motor features according to whether they are triggered by emotional or voluntary responses [41–46], and it may be that the two contexts focus on different types of startle.

Direct testing of dogs in the behavioural test may be more reliable, but is more labour intensive, especially when relying on clinical cases. The need to design a behavioural test without compromising animal welfare meant that the chosen stimuli were not so intense as to scare or seriously threaten the animal. As a consequence, the magnitude of the behavioural responses that we observed may have been less compared to reactions that the animal in treatment might have exhibited in real life fearful or threatening conditions. This could explain why the effects in the test seemed relatively mild when compared to the survey results. The findings of the surveys and the tests provide convergent validity that, together with a consistency at the theoretical level, indicate that these results are robust and the effects reliable.

5. Conclusion

Overall, these results indicate that in pet dogs, corticosteroid treatment at therapeutic doses can bias cognition and change behaviour.

Physiological intervention with these drugs appears to increase sensitivity towards aversion. On the basis of these results and in the absence of evidence to the contrary, we recommend that the supply of these drugs to owners by veterinarians should be accompanied by advice about behavioural risk management due to a possible increase of a negative affective state, a condition that might increase the risk of aggressive behaviours.

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